A STUDY OF THE *B. COLI* GROUP WITH SPECIAL REFERENCE TO THE SEROLOGICAL CHARACTERS OF THESE ORGANISMS.

By T. J. Mackie.

Introductory Discussion: The Classification of "Coliform" Bacilli and the Biological Relationships of the *B. coli* Group to other Gram-Negative Aërobic Intestinal Bacilli.

In 1885 Escherich recorded the isolation of a Gram-negative intestinal bacillus which he designated *B. coli communis*, and this classical type has been long considered the prevalent intestinal bacterium. In general this organism has been described as a motile, non-sporing, aërobic, facultatively anaërobic, Gram-negative bacillus, which grows at 37° C., ferments glucose, lactose and dulcite with acid and gas production, forms indol from peptone, clots milk, reduces nitrates, and grows in gelatin media without producing liquefaction.

With the advance of bacteriological knowledge and as a result of extensive investigations on the bacteriology of faeces, water, milk, etc., this original type has come to represent only one of a considerable bacterial class or group. The organisms of this group, however, are characteristic normal inhabitants of the animal intestine, and are therefore conveniently designated by the generic term *B. coli*.

Their pathological importance has been well established in virtue of their potentially pathogenic properties, and in the bacteriological diagnosis of intestinal infections their precise recognition and the separation and differentiation of specific pathogenic organisms from them have necessitated the most careful study of their biological characters. Moreover their occurrence in water supplies has led to an extensive study of the whole group from the point of view of sewage contamination, and the importance of certain types as indicators of such contamination has been emphasised. Thus, the detection of these organisms constitutes an essential method in the routine examination of water supplies. This group of bacteria is also concerned in the process of souring of milk, and certain of the classical types were originally isolated from this source, *e.g. B. acidi lactici* (Hüppe).

While the whole group of organisms biologically allied to Escherich's

original type has been classified as *B. coli*, the question as to which varieties represented "typical" *B. coli* as apart from "atypical" forms has been studied in connection with the bacteriology of water (Houston and others). Much confusion has resulted, however, as regards the definition of a typical *B. coli* owing to the different identification standards set up by various observers. Some of these definitions have been too limited in their application and different degrees of importance have been attached to different characters (Savage, Prescott and Winslow, Konrich, Reports of the English and American Committees on standard methods for the examination of water).

While there has been considerable variation in the criteria adopted by different workers for the identification of the colon bacillus, the characters generally accepted as common to the whole group of "*B. coli*" may be summed up as follows: Gram-negative, aërobic, facultatively anaërobic non-sporing bacilli growing at 37° C., and fermenting glucose and lactose. Other characters, *e.g.* different fermentative reactions, motility, indol production, Voges and Proskauer reaction, simply determine the differentiation of separate types. Thus the term "lactose-fermenter" has been frequently used as a convenient designation for the group (MacConkey).

The question arises as to whether gelatin-liquefying, Gram-negative bacilli which correspond in other characters to *B. coli* types are to be included in this biological group. Among the lactose-fermenters classified by MacConkey certain gelatin liquefiers were represented, *e.g. B. cloacae, B. oxytocus perniciosus*, and other authorities (Prescott and Winslow) have accepted this reaction as one of the possible characters of the group. In my own experience of a large number of coliform strains from faeces, urine, etc., and also water (Egypt, 1915–1918) I have never met with gelatin-liquefying, Gram-negative, aërobic, non-sporing bacilli capable of growing at 37° C. apart from those referable to the *B. proteus* type.*

While the characteristic *B. coli* has the property of fermenting lactose, it is doubtful (as will be shown later, p. 350) if lactose fermentation can be regarded as a specific character of a particular group of intestinal bacilli, and studies on the variation of these organisms (*v.* p. 360) have shown how certain types only acquire this property by mutation on culture medium.

Thus non-gelatin-liquefying, glucose-fermenting, non-lactose-fermenting bacilli of intestinal origin have to be considered in any system of classification of the Gram-negative intestinal bacilli.

From our present knowledge of the aërobic intestinal bacilli it would appear more rational from the purely biological standpoint to recognise a

* *B. proteus*: Gram-negative, motile, aërobic, non-sporing bacilli, growing well at 37° C., showing a tendency to "spreading" type of growth, fermenting glucose with or without gas, not fermenting lactose, dulcite or mannite, varying in fermentation of saccharose and in indol formation, liquefying rapidly gelatin or solidified serum.

large class of intestinal bacilli having the common characters: aërobic, Gram-negative, non-sporing, growing at 37° C., fermenting glucose with or without gas production, not liquefying gelatin; comprising certain specific pathogenic organisms specially designated, and identified by cultural and serological characters, *e.g. B. typhosus*, etc., and a large number of saprophytic, though potentially pathogenic, varieties which can be generally classified into different types according to their cultural characters. The term *B. coli* (if it is to be used at all) in its widest application would thus become referable to those members of the class which do not possess *specific* pathogenic properties irrespective of certain cultural characters, including lactose fermentation. The term "typical *B. coli*" would be used, if necessary, to designate those types (fermenting lactose, clotting milk, and producing indol) which are most prevalent in the intestine, and therefore undoubted indicators in water of recent sewage pollution. The only justification for recognising a special group of "lactose fermenters" depends on the statistical fact that these types are most prevalent in fresh animal excreta, but it is questionable if the statistical basis can be used for a biological classification.

The further question arises as to the significance of gelatin liquefaction, and whether a wider definition of the class is required, *i.e.* to include *B. proteus*. It must be admitted that with the exception of gelatin liquefaction, types of *B. proteus* may correspond closely to certain non-liquefying intestinal bacilli.

Organisms of the *B. faecalis alkaligenes* type have also to be considered in regard to the classification of the Gram-negative intestinal bacilli. *B. faecalis alkaligenes* has been long recognised as differing from the motile organisms of the coli-typhoid class in the "terminal" position of its flagella, as opposed to the "lateral" arrangement in the case of *B. coli* and *B. typhosus* (Berghaus, Klimenko). While this is true for my own observations of these organisms, non-motile, Gram-negative, aërobic bacilli which do not ferment glucose or any other sugar have been frequently noted in dysenteric stools, and strains of this type have been found which on first isolation did not ferment glucose, and only gained this property after a period of growth in a glucose medium. This might appear to link up organisms of the *B. faecalis alkaligenes* type with the sugar-fermenting intestinal bacilli.

The "lactose-fermenters" have been the subject of much careful study: originally different classical types had been separately described, *e.g. B. coli communis*, *B. neapolitanus* (Emmerich), *B. acidi lactici* (Hüppe), *B. lactis aërogenes* (Escherich), etc., and these names may still be retained for organisms which accurately correspond to such classical strains.

The work of MacConkey on the lactose-fermenting coliform bacilli represented the first attempt in the direction of a rational system of classification of these organisms.

A number of cultural tests had been commonly employed which were shown by him to be of little differential value, and he further established the importance of a selected though comprehensive series of biochemical reactions as type criteria.

MacConkey (1905), in his first paper dealing with this subject, arbitrarily divided the lactose-fermenters into four sub-groups according as they did or did not decompose saccharose and dulcite. The first group, represented by the classical *B. acidi lactici*, included those which fermented neither dulcite nor saccharose; the second included those which fermented dulcite but not saccharose, *e.g. B. coli communis*; the third comprised types such as *B. neapolitanus* which fermented both dulcite and saccharose; and the fourth consisted of strains which fermented saccharose, but not dulcite.

This classification was of course entirely arbitrary and incomplete.

In 1909 MacConkey reviewed the whole subject, and indicated that if, in addition to the fermentation of dulcite and saccharose, further tests were added—effect on adonite and inulin, presence of motility, indol production, the Voges and Proskauer reaction—theoretically 128 possible varieties could be differentiated. At that time he had examined 497 strains from human and animal faeces, water, etc., and of the 128 possible types had met with 36 varieties differentiated according to their action on (1) gelatin, (2) dulcite, (3) saccharose, (4) adonite, (5) inulin, in some instances, (6) inosite, and by (7) the presence or absence of motility, (8) indol production and (9) the Voges and Proskauer reaction.

MacConkey had at the same time tested a number of other fermentable substances which had been commonly employed, but indicated that no further information was to be obtained by the use of sugars, etc., such as galactose and laevulose, on which the various sub-groups had all the same effect; and in the case of quercite and erythrite found that practically none of his strains had any fermentative action. Out of 497 strains examined 178 were from human faeces, and of the various types noted the most prevalent were type No. 71, *B. coli communis*, and *B. vesiculosus* (see Table I).

MacConkey's system of classification has been supported and adopted by Bergey and Deeham, Clemesha and others, and modified by Jackson, who employed the reactions in mannite and raffinose as further differential characters.

Howe, on the other hand, claimed that motility, indol formation, mannite and dulcite fermentation were of little value for classification owing to the fact that, from the statistical point of view, these reactions showed no correlation with one another or with other criteria. Prescott and Winslow have also urged the value of the statistical basis for a biological classification, and that the characters of these organisms should be considered not independently, but in relationship to one another.

TABLE I. (*Quoted from MacConkey*, 1909.)

Type (MacConkey's classification).	Number of strains.	Motility.	Lactose.	Dulcite.	Saccharose.	Adonite.	Inulin.	Inosite.	Indol.	Voges and Proskauer reaction.	Gelatin.
No. 1	9	+	+	—	—	+	—	—	+	—	—
B. acidi lactici (Hüppe), No. 2	1	—	+	—	—	+	—	—	+	—	—
B. Grünthal, No. 4	8	+	+	—	—	—	—	—	+	—	—
B. vesiculosus, No. 5	33	—	+	—	—	—	—	—	+	—	—
No. 7	1	+	+	—	—	—	—	—	—	—	—
B. coli mutabilis, (Massini) No. 8	—	—	+	—	—	—	—	—	—	—	—
No. 33	—	+	+	+	—	+	—	—	+	—	—
B. coli communis, No. 34	37	+	+	+	—	—	—	—	+	—	—
B. Schafferi, No. 35	11	—	+	+	—	—	—	—	+	—	—
No. 67	1	—	+	+	+	+	—	+	—	+	—
No. 71	42	+	+	+	+	—	—	—	+	—	—
B. neapolitanus, No. 72	15	—	+	+	+	—	—	—	+	—	—
No. 74	1	+	+	+	+	—	—	—	—	—	—
No. 75	—	—	+	+	+	—	—	+	—	+	—
No. 99	—	—	+	—	+	+	+	+	—	—	—
No. 101	1	—	+	—	+	+	—	+	+	—	—
B. lactis aërogenes, No. 103	8	—	+	—	+	+	—	+	—	+	—
No. 106	2	+	+	—	+	—	—	—	+	—	—
B. coscoroba, No. 107	1	—	+	—	+	—	—	—	+	—	—

(In this and subsequent tables + = acid and gas, liquefaction of gelatin, motile, indol production, as the case may be; ⊥ = acid without gas; — = no acid or gas production, etc.)

Kligler emphasised the importance of salicin fermentation for differential purposes, and elaborated a comprehensive system of classification of dextrose-fermenting intestinal bacilli; he subdivided these organisms first according to their action on lactose, and the further classification depended mainly on the reactions in dulcite, saccharose and salicin; he included among the various sub-groups *B. proteus* and *B. cloacae* (gelatin liquefiers).

An older system of classification which is of interest to consider briefly is that of Jensen, who, on the basis of certain simple fermentative reactions, arbitrarily divided the whole coli-typhoid group into several main sub-groups; the criteria were the reactions in glucose, lactose, saccharose and maltose, and in this way the following organisms were differentiated: *B. faecalis alkaligenes*, *B.* "*metacoli*," *B. typhosus*, *B. paracolon*, *B. coli anaerogenes*, *B. coli* (two types) and *B.* "*pseudocoli*." This system was later amplified for the differentiation of coliform bacilli by Wulff, who employed certain additional tests—galactose, glycerin, adonite, mannite, dulcite and xylose fermentation.

This system of classification cannot be considered as sufficiently complete; thus no cognisance was taken of indol production and the fermentation of inosite. As will be shown later (*v.* p. 347), these reactions are important and characteristic features of certain *B. coli* sub-groups.

Houston, in connection with the bacteriological examination of water, has classified coliform bacilli into "typical" and "atypical" varieties (*v. supra*), and further divided the typical organisms according to the fermentation of dulcite and saccharose.

While different systems of classification have thus been adopted which in the hands of various workers have served a practical purpose in enabling them to recognise typical varieties as apart from those which are less characteristic, it can hardly be claimed that these organisms have yet been completely classified, nor that the significance of different characters has been accurately assessed.

General Outline of the Investigation.

In the observations to be recorded a further study has been made of the "*B. coli*" group, and the characters of a large number of strains of Gram-negative, aërobic, non-sporing, glucose-fermenting, non-liquefying bacilli (excluding the specific pathogenic organisms of this class) have been investigated.

The question of the biological classification of these organisms has also been studied from the serological aspect.

In addition certain observations have been made with regard to variations among the coliform bacilli and their biological significance.

The various strains were isolated from pathological specimens of urine, faeces, appendix, abscesses, etc.

Criteria Employed for Differentiation of Types.

In classifying coliform bacilli into particular types MacConkey's criteria were for the most part adopted, and for all practical purposes proved sufficiently complete. Raffinose and salicin fermentations were, however, found to yield further differentiation, and these tests were therefore included among the criteria adopted.

Certain other fermentable substances which have been employed by different workers proved of little or no differential value; thus maltose was found to be fermented by all the lactose-fermenting strains; glucose-fermenting, non-lactose-fermenting strains, however, vary in their action on this sugar. The same was found to be true for mannite. Laevulose and galactose were with few exceptions fermented by all the glucose-fermenting strains and glycerin by all the lactose-fermenters. Of course, as is well known, all lactose fermenters are also glucose fermenters. The behaviour of these bacilli in raffinose in the majority of cases corresponded to their effect on saccharose (as shown also by Winslow and Walker), but a certain proportion exhibited differences in their effects on these sugars.

The action of the lactose fermenters on dextrine differs from the fermentation produced in other carbohydrates; in the case of simple peptone water media containing dextrine and neutral red (as indicator), it was found that after twenty-four hours' incubation the neutral red became of a bright yellow colour and there was some degree of gas production; the gas formed was analysed and found to consist mainly of hydrogen. The medium remained neutral. This effect was common to all the lactose-fermenting types and the test afforded no information as regards differentiation of various strains.

With one exception all the strains that fermented inosite also fermented adonite. In all cases the Voges and Proskauer reaction was only given by inosite-fermenting strains.

It was found on repeated testing of various strains that the presence (or absence) of motility was a definite and constant character.

Similarly the presence (or absence) of indol in peptone water cultures (after ten days at 37° C.) proved to be a stable property of these organisms.

It was concluded therefore that of the various tests used by different workers, the most complete set of criteria for the differentiation of *B. coli* types were: (1) Presence or absence of motility; (2) production of indol; (3) the Voges and Proskauer reaction; the fermentation of (4) glucose, (5) lactose, (6) dulcite, (7) saccharose, (8) adonite, (9) inulin, (10) inosite, (11) raffinose, (12) salicin.

The majority of the strains to be described were re-tested after about two or three months, and apart from the variations noted (p. 360) their characters proved markedly stable.

Methods.

Motility.—In determining the presence or absence of this character a hanging-drop preparation from a 4 to 6 hours bouillon culture, or the "condensation" fluid of a sloped-agar culture of the same age, was examined. Tested in this way the presence (or absence) of motility proved to be a definite character.

Liquefaction of Gelatin.—This was tested for by making stab inoculations in ordinary nutrient gelatin and incubating the tubes at 22° C. for two weeks.

Indol Production.—The presence or absence of this property was determined by testing a ten-days peptone water culture of the strain in question with Ehrlich's reagents, paradimethylamidobenzaldehyde and persulphate of potassium, according to the usual procedure.

Voges and Proskauer Reaction.—For this test a 2 per cent. peptone water solution containing 1 per cent. glucose was inoculated and incubated at 37° C. for three days, when a solution of potassium hydrate was added and the tube allowed to stand at room temperature for several hours. A positive reaction was indicated by the development of a red fluorescence.

Fermentation Test.—The basis of the medium used for testing sugar fermentations was a 2 per cent. peptone solution with 0·5 per cent. sodium chloride. To avoid the possible decomposition of the sugar in the medium by overheating in the process of sterilisation, the different fermentable substances were added to the already sterilised medium in the form of sterile watery solutions. The proportion of sugar in the medium was 1 per cent.; neutral red (0·25 per cent. of a 1 per cent. watery solution) was added as an indicator of acid production. The medium was distributed in Durham's tubes (for the observation of gas production), and placed at 100° C. in the Koch's steriliser for ten minutes on two successive days. This short final sterilisation, while not acting deleteriously on the sugar, was sufficient to ensure complete sterility.

I have observed that for the proper appreciation of gas production fluid media must be used, as shake or stab cultures in solid agar media are open to fallacy. With certain specimens of peptone water agar (without meat extract), it was found that gas production might result on inoculation with *B. coli*, *i.e.* even in the absence of sugar, and this was especially marked if the agar was not freshly prepared.

Thus, a shake culture of *B. coli communis* made in peptone water agar immediately after preparation showed no gas production in the medium; the same medium a week later was again inoculated and on this occasion a considerable number of gas bubbles appeared in the medium after twenty-four hours' incubation; a fortnight later inoculation resulted in an abundant production of gas throughout the medium. No acid formation was, however, noted.

Litmus has been frequently used as an indicator of acid formation, but with this agent, especially when incorporated in nutrient medium, difficulties have frequently arisen owing to the many intermediate degrees of colour between unequivocal alkalinity on the one hand and undoubted acidity on the other. It has the further disadvantage, as I have noted, of being decolorised rapidly by many organisms of the *coli* group. In my own experience neutral red is a more valuable indicator as it is not subject to these disadvantages.

System of Classification and Designation Adopted.

Among the coliform bacilli studied, it seemed possible to classify into separate sub-groups, types of organisms having well-defined common characters.

Thus the different types which were characterised by the absence of gas production in the case of all the sugars fermented even after repeated sub-culture (*coli anaërogenes*) were classified in a separate sub-group. Serological investigations (*v. infra*) in which the group action of the complement-deviating antibody of immune sera to certain more typical *B. coli* varieties showed that the *coli anaërogenes* organisms were not closely related to the gas-producing types of *B. coli*.

It was also noted that certain types of coliform bacilli which produced on culture medium unusually large, thick, opaque, slimy and sometimes "viscid" colonies were all characterised by possessing the power of fermenting inosite while the usual *B. coli* varieties had not this property. These organisms were therefore grouped together and classified apart from the more typical coliform bacilli. All these varieties proved to be non-motile, all fermented lactose, saccharose, raffinose and salicin, and with one exception adonite; in this sub-group therefore several characters were definitely correlated. The separation of these organisms from the typical coliform bacilli was also found to be justified by serological tests as in the case of the *anaërogenes* sub-group.

It was further concluded from the serological studies referred to above, and dealt with in detail later, that a sub-group comprising all the "typical *B. coli*" could be recognised whose common characters were—gas-producing, indol-forming, non-inosite-fermenting.

After defining these three sub-groups, there still remained the organisms having the common characters—gas-producing, non-inosite-fermenting, non-indol-forming. Serological observations showed that this category did not represent a serologically homogeneous sub-group, but for purposes of a preliminary classification I have grouped all the organisms of the category together.

Thus four main sub-groups of the Gram-negative, glucose-fermenting, non-gelatin-liquefying intestinal bacilli may be recognised and this system of classification has been used in the following records. In addition, the

TABLE II.—*Sub-group A: Gas producing, indol forming, non-inosite fermenting, non-liquefying.*

Type.	Motility.	Glucose.	Lactose.	Dulcite.	Saccharose.	Adonite.	Inulin.	Raffinose.	Salicin.	Proskauer reaction.	Mannite.	Maltose.	Faeces.	Urine.	Appendix abscesses	Other sources.	Total.
1. MacConkey, No. 71	+	+	+	+	+	—	—	+	+	—			10	15	3		28
2. *B. coli communis*	+	+	+	+	—	—	—	—	+	—			14	5	1	1 (Blood culture)	21
3. *B. vesiculosus*	—	+	+	—	—	—	—	—	—	—			4	5	4		13
4. *B. Grünthal*	+	+	+	—	—	—	—	—	+	—			4	6	2		12
5. *B. Schafferi*	—	+	+	+	—	—	—	—	+	—			4	6	1		11
6. *B. neapolitanus*	—	+	+	+	+	—	—	+	+	—			2	3		1 (Puerperal fever)	6
7. *B. Schafferi* type	—	+	+	+	—	—	—	—	—	—			1	3	2		6
8. MacConkey, No. 71 type	+	+	+	+	+	—	—	+	—	—			2	1	1	1 (Conjunctivitis)	5
9. MacConkey, No. 106	+	+	+	—	+	—	—	+	+	—			1	4			5
10. Neapolitanus type	—	+	+	+	+	—	—	+	—	—			1	2	1		4
11. MacConkey, No. 1	+	+	+	—	—	+	—	—	+	—			4				4
12.	—	+	—	+	—	—	—	—	—	—	+	+	4				4
13. *B. coscoroba*	—	+	+	—	+	—	—	+	+	—			2		1		3
14. *B. vesiculosus* type	—	+	+	—	—	—	—	—	+	—				2	1		3
15.	+	+	+	+	+	+	—	+	+	—			1	1		1 (Otitis)	3
16. *B. coli communis* type	+	+	+	+	—	—	—	+	+	—			1	2			3
17. *B. coli communis* type	+	+	+	+	—	—	—	—	—	—			1	2			3
18. *B. Schafferi* type	—	+	+	+	—	—	—	+	+	—			1	1			2
19.	—	+	—	+	+	—	—	+	—	—	+	+	1	1			2
20. *B. MacConkey*, No. 106 type	+	+	+	—	+	—	—	—	+	—			1	1			2
21. Neapolitanus type	—	+	+	+	+	—	—	—	+	—			1	1			2
22. MacConkey, No. 71 type	+	+	+	+	+	—	—	—	+	—						1 (Conjunctivitis)	1
23. *B. vesiculosus* type	—	+	+	—	—	—	—	+	—	—			1				1
24. *B. coli communis* type	+	+	+	+	—	—	—	+	—	—			1				1
25. *B. Grünthal* type	+	+	+	—	—	—	—	+	+	—				1			1
26. *B. Grünthal* type	+	+	+	—	—	—	—	—	—	—			1				1
27. MacConkey, No. 1 type	+	+	+	—	—	+	—	—	—	—				1			1
28. *B. acidi lactici*	—	+	+	—	—	+	—	—	—	—			1				1
29.	+	+	—	+	—	—	—	—	—	—	+	+	1				1
30.	—	+	+	+	+	+	+	+	+	—			1				1
31. MacConkey, No. 33	+	+	+	+	—	+	—	—	+	—			1				1
32.	—	+	+	—	+	—	+	+	+	—					1		1
33.	—	+	+	—	+	+	—	+	—	—			1				1
34.	—	+	—	—	+	—	—	—	+	—	+	+		1			1
35.	—	+	—	+	+	—	—	—	+	—	+	+		1			1
36.	+	+	—	—	—	—	—			—	—	—		1			1
											Total		68	66	18	5	157

TABLE III.—*Sub-group B: Gas producing, non-indol forming, non-inosite fermenting, non-liquefying.*

Type.	Motility.	Glucose.	Lactose.	Dulcite.	Saccharose.	Adonite.	Inulin.	Raffinose.	Salicin.	Proskauer reaction.	Mannite.	Maltose.	Faeces	Urine.	Appendix abscesses.	Other sources.	Total
1. *B. MacConkey*, No. 74	+	+	+	+	+	—	—	+	+	—			4	8		1 (Cholecystitis)	13
2.	—	+	+	+	+	—	—	+	+	—			2	4			6
3.	—	+	+	+	—	—	—	—	+	—			1	1			2
4. *B. MacConkey*, No. 8 .	—	+	+	—	—	—	—	—	—	—			1		1		2
5. *B. MacConkey*, No. 8 .	—	+	+	—	—	—	—	—	+	—			1	1			2
6.	+	+	+	—	+	—	—	+	+	—				1			1
7.	—	+	+	—	+	—	—	+	+	—				1			1
8.	—	+	+	+	—	—	—	—	—	—				1			1
9.	+	+	+	—	+	—	—	+	—	—				1			1
10.	—	+	+	+	+	+	—	+	+	—				1			1
11. *B. MacConkey*, No. 7 .	+	+	+	—	—	—	—	—	—	—			1				1
101. *B. paracolon* type .	+	+	—	—	—	—	—			—	+	+	2	4			6
102.	+	+	—	+	—	—	—	—	+	—	+	+	5				5
103. *B. paracolon* type .	—	+	—	—	—	—	—			—	+	+	2	3			5
104.	+	+	—	—	+	—	—	+	+	—	+	+	2				2
105.	+	+	—	—	+	—	—	+	—	—	+	+	1				1
106.	—	+	—	—	+	—	—	+	—	—	+	+		1			1
107.	—	+	—	+	+	—	—	+	+	—	+	+		1			1
											Total .		22	28	1	1	52

TABLE IV.—*Sub-group C: Gas producing, inosite fermenting, non-liquefying.*

Type.	Motility.	Glucose.	Lactose.	Dulcite.	Saccharose.	Adonite.	Inulin.	Raffinose.	Salicin.	Proskauer reaction.	Indol.	Fæces.	Urine.	Appendix abscesses.	Other sources.	Total.
1. *B. MacConkey*, No. 67	—	+	+	+	+	+	—	+	+	+	—	3	2			5
2.	—	+	+	+	+	+	+	+	+	+	+	3				3
3.	—	+	+	+	+	+	+	+	+	—	+	3				3
4.	—	+	+	+	+	+	—	+	+	—	+	3				3
5. *B. lactis aërogenes*	—	+	+	—	+	+	—	+	+	+	—	2			1 (Skin ulcer)	3
6. *B. MacConkey*, No. 99	—	+	+	—	+	+	+	+	+	—	—	1	1			2
7.	—	+	+	—	+	+	+	+	+	+	+	1	1			2
8.	—	+	+	+	+	+	—	+	+	+	+	1		1		2
9. *B. MacConkey*, No. 101	—	+	+	—	+	+	—	+	+	—	+	1	1			2
10.	—	+	+	—	+	+	—	+	+	—	—	1				1
11. *B. MacConkey*, No. 75	—	+	+	+	+	—	—	+	+	+	—	1				1
										Total		20	5	1	1	27

TABLE V.—*Sub-group D: Non-gas producing in all Sugars fermented, non-liquefying.*

Type.	Motility.	Glucose.	Lactose.	Dulcite.	Saccharose.	Adonite.	Inulin.	Inosite.	Raffinose.	Salicin.	Proskauer reaction.	Indol.	Mannite.	Maltose.	Fæces.	Urine.	Other sources.	Total.
1.	—	+	—	—	—	—	—	—			—	—	+	+		2	1 (Skin ulcer)	3
2.	—	+	—	—	—	—	—	—			—	+	+	+		1		1
3.	+	+	+	—	+	—	—	—			—	+	+	+	1			1
4.	—	+	+	—	+	—	—	—			—	+	+	+		1		1
5.	—	+	+	—	—	—	—	—			—	+	+	+		1		1
6.	—	—	+	—	—	—	—	—			—	—	+	+		1		1
7.	—	+	—	+	+	+	—	—	—	+	—	+	+	+		1		1
8.	—	+	—	—	+	+	—	—	—	+	—	—	+	+		1		1
												Total			1	8	1	10

various types belonging to these sub-groups have been designated numerically (*i. e.* 1, 2, 3, 4, etc.) in order of their prevalence in the series investigated. In the *B.* category the lactose-fermenters (1 to 11) have been distinguished from the non-lactose-fermenters (101 to 107) by a different series of numbers. The latter class includes certain "paracolon" types which in the serological tests appeared to be differentiated from organisms fermenting lactose in primary culture.

Two hundred and forty-six strains from urine (pathological specimens), faeces and other sources have been carefully studied and classified in this way (Tables II, III, IV, V). In Table VI, for comparison, those strains which correspond to MacConkey's types are classified according to his system.

Table VI.

MacConkey's types.	Faeces.	Urine.	Appendix Abscesses.	Other Sources.	Total.
No. 1	4	1	—	—	5
No. 2. *B. acidi lactici*	1	—	—	—	1
No. 4. *B. Grünthal*	5	7	2	—	14
No. 5. *B. vesiculosus*	5	7	5	—	17
No. 7	1	—	—	—	1
No. 8	2	1	1	—	4
No. 33	1	—	—	—	1
No. 34. *B. coli communis*	17	9	1	1	28
No. 35. *B. Schafferi*	6	10	3	—	19
No. 67	3	2	—	—	5
No. 71	12	16	4	2	34
No. 72	4	6	1	1	12
No. 74	4	8	—	1	13
No. 75	1	—	—	—	1
No. 99	1	1	—	—	2
No. 101	1	1	—	—	2
No. 103. *B. lactis aërogenes*	2	—	—	1	3
No. 106	2	5	—	—	7
No. 107. *B. coscoroba*	2	—	1	—	3
				Total	172

Note.—Of 246 strains 172 corresponded to types described by MacConkey.

Types of Coliform Bacilli Found in Pyogenic Infection of the Urinary System.

As shown by MacConkey, certain coliform types tend to be more prevalent than others in human and animal faeces; thus the type designated by him No. 71 is of commonest occurrence. As is well known, Gram-negative bacilli are the most frequent causative agents in pyogenic infections

of the urinary tract, and are found either alone or associated with the pyogenic cocci. In order to ascertain what types were characteristic of these pathological conditions a number of coliform bacilli isolated from urinary cases were carefully investigated.

The specimens of urine were mainly from cases of cystitis and pyelitis. The samples were centrifugalised in sterile tubes, and from the sediment, plates of Endo-agar or MacConkey's bile-salt-neutral-red-lactose-agar were inoculated by successive strokes of the platinum loop. Separate colonies were thus obtained, and to ensure the purity of the ultimate culture investigated further successive strokes were generally made (from single colonies) on another plate, and the final culture made from a single colony on the second plate. In some cases agar slope cultures were made from single colonies on the primary plate; this culture was then replated and the final culture obtained by subinoculating a single colony.

One hundred and seven strains of coliform bacilli from 90 cases of urinary sepsis were examined. In the majority of instances pure cultures of individual types were isolated. It might be expected in dealing with a bacterial group such as *B. coli* that separate colonies similar in appearance might on examination prove to belong to different types. I have on several occasions selected two or three similar colonies at random from the same plate and found on examination that they represented the same cultural type. Not infrequently, however, mixed cultures are met with, but it is remarkable that in such instances one notices some difference in the colonies, *e.g.* difference in size, thickness, depth of colour on Endo or MacConkey's agar, or complete absence of pink coloration. Of course non-lactose-fermenters produce "pale" colonies on these differential media, but in primary culture the absence of red coloration is no proof that the organism is a non-lactose fermenter. The fact that mixed cultures of different coliform species may occur in urinary sepsis is of special and obvious interest as regards the preparation of autogenous vaccines.

As in the case of faecal strains there is a distinct tendency towards the prevalence of certain types in urinary conditions, and the commonest intestinal species are also the most frequent types met with in pathological urines.

Type 1, Sub-group A, corresponds to *B. MacConkey* No. 71 and is the commonest coliform species met with (*v.* Tables I, II, VI). It proved the most frequent type (15 strains) in urinary sepsis. The other prevalent varieties were types A2 (*B. coli communis*), A3 (*B. vesiculosus*), A4 (*B. Grünthal*) A5 (*B. Schafferi*), B1 (*B. MacConkey* No. 74).

Of the 107 strains—

66 belonged to the sub-group A.
28 belonged to the sub-group B.
5 belonged to the sub-group C.
8 belonged to the sub-group D.

Thus the majority are referable to the A sub-group, which comprises all the "typical" *B. coli* varieties. Strains of non-lactose-fermenters were also isolated, A19, A34, A35 and A36; those of types 19, 34 and 35, however, developed lactose-fermenting variants in lactose medium (*v.* Table XXIX).

Type A36 corresponded in cultural reactions to *B. Morgan* No. 1; it was isolated in pure culture from a case of pyelitis and cystitis.

In sub-group B, type 1 was of commonest occurrence, corresponding to MacConkey's type No. 74. The relative prevalence of other varieties is shown in Table III. Among the organisms of sub-group B certain non-lactose-fermenters are also represented, including 7 strains of "paracolon" types B101 and B103.

Patrick reported organisms of these types in cases of bacilluria occurring in the course of typhoid fever. They were present in such large numbers as to render the urine turbid. In primary culture they did not exhibit any gas production and therefore simulated *B. typhosus* in cultural reactions. I had the opportunity of examining his strains, and found them to be typical "paracolon" bacilli with the reactions shown (B101 and 103) and similar to strains I had isolated from cases of urinary sepsis.

Certain non-lactose-fermenters of the B sub-group were also noted which developed lactose-fermenting variants spontaneously in lactose media (B106 and 107) (*v.* Table XXIX).

Five inosite-fermenters (sub-group C) were isolated from urine, and 8 strains referable to sub-group D. Four of the latter were non-lactose-fermenters; 2 of these fermented only glucose, and, being non-motile, were therefore similar in cultural reactions to *B. dysenteriae*, Shiga. They were not, however, agglutinated by an anti-Shiga serum. One of these strains developed a lactose-fermenting variant. The characters of these strains are shown in Table V.

Among the Gram-negative bacilli found in these cases of urinary sepsis *B. proteus* (*Urobacillus liquefaciens septicus Krogius*) was not infrequently noted, usually associated with *B. coli* or pyogenic cocci; Gram-negative bacilli characterised by absence of carbohydrate fermentation have also been noted both in mixed and pure culture; 4 strains of this type were isolated; 2 were motile (corresponding to the typical *B. faecalis alkaligenes*) and 2 non-motile.

Types Isolated from Faeces, Appendix Abscesses and other Sources.

One hundred and eleven strains from specimens of faeces were analysed in the same way; these were derived partly from normal specimens and also specimens submitted for bacteriological examination, *e. g.* for detection of *B. typhosus*. Their grouping was as follows:

Sub-group A, 68.
Sub-group B, 22.
Sub-group C, 20.
Sub-group D, 1.

As in the case of the urinary strains most belonged to the sub-group A; only one *anaërogenes* type was noted (D3).

The majority of the strains classified in this series were isolated from plates made directly from faeces, but 4 of sub-group B and 10 of C were obtained from cultures in brilliant green peptone water employed for the enrichment of *B. typhosus* (Browning, Gilmour and Mackie). As indicated later it was found that types of B and C sub-groups were more resistant to brilliant green than the A types; hence the relatively large proportion of C types in the series.

Among these strains were 4 non-lactose-fermenters of the A12 type and 1 of the A29 type; these two varieties differ from one another in motility. One non-lactose-fermenter of the A19 type was also isolated. This strain, like the similar type found in urine, developed a lactose-fermenting variant (*v.* Table XXIX).

Five non-lactose-fermenters of the B102 type were isolated from faeces, but all of these developed lactose-fermenting mutants. The other non-lactose-fermenters noted were B101 (2 strains), B103 (2 strains), B104 (2 strains), and B105. Single strains of the B103 and B105 types developed lactose-fermenting variants (*v.* Table XXIX).

Twenty strains from appendix abscesses were also investigated; these were grouped as follows:

Sub-group A, 18, of which the A3 and A1 types were most prevalent.
(All were lactose-fermenters [*v.* Table II].)
Sub-group B, 1.
Sub-group C, 1.

A few strains from other sources were examined: 2 from cases of conjunctivitis both belonging to the A sub-group, 1 from a suppurative otitis (A15), 1 from a case of puerperal sepsis (A6), 2 from skin ulcers (C5 and D1), 1 from a case of cholecystitis (B1), 1 from blood-culture after an abdominal operation (A2).

The total number of strains examined from different sources could therefore be classified as follows:

Sub-group A, 157.
(A1 most prevalent type, 28 strains;
10 primarily non-lactose-fermenters.)
Sub-group B, 52
(B1 most prevalent, 13 strains;
21 primarily non-lactose fermenters.)
Sub-group C, 27.
(C1 most prevalent type, 5 strains.)
Sub-group D, 10.
(D1 most prevalent type, 3 strains.)

Considering the various strains collectively in these series which corre-

spond to types isolated and classified by MacConkey, the commonest is the No. 71 type (34 strains) (Table VI). Of the series isolated from urine this type was also the most prevalent, though among the faecal strains the *B. coli communis* was the commonest. Collectively, however, the most prevalent types in my series were *B. MacConkey* No. 71 and *B. coli communis*.

With comparatively few exceptions, the Gram-negative bacilli found in urinary sepsis, as shown, are normal inhabitants of the intestine, and this parallelism between the faecal and urinary types is a further confirmation of the generally accepted view that these infections are auto-infections from the bowel.

As indicated above, the A sub-group comprises the most frequently occurring types of coliform bacilli, the so-called typical varieties. This group also appears to embrace the largest number of different varieties or types (36). While organisms of the sub-groups B and C are less frequently met with in excremental material, they nevertheless represent characteristic faecal organisms. Organisms of the *anaërogenes* type are of comparatively rare occurrence, but, as shown, this type may be associated with certain cases of urinary sepsis.

The Serological Investigation of the *B. coli* Group.

The following serological studies were originally carried out with a view to throwing further light on the biological relationships of the various types of *B. coli* and its congeners. The agglutination and complement-deviation reactions of immune sera to certain of the commoner varieties were studied, and the results, apart from their bearing on this particular question, represent observations of considerable interest from the purely immunological standpoint.

Agglutination Reactions.

While in the case of *B. typhosus*, precise species specificity is characteristic of an agglutinating antiserum, among the coliform bacilli a much more restricted degree of specificity was observed—specificity for the individual strain.

An immune serum to a particular strain of *B. typhosus* will agglutinate most *B. typhosus* strains with little variation in degree; immune sera to certain *B. coli* types, on the other hand, have been found to exert little or no action on other strains identical as regards cultural reactions to that used for immunisation.

Several authors have drawn attention to the high degree of specificity of *B. coli* agglutinins (Van Everen, Pfaundler, Cany, Wolf, Amiradzibi), but the agglutination reactions of these organisms have not been accurately studied in correlation with their classification into different types *qua* cultural reactions; thus Amiradzibi immunised guinea-pigs against five strains which

all possessed certain common characters—power of fermenting glucose, lactose, clotting milk and producing indol; he found that the strain used for immunisation was the only one agglutinated. These common characters are, however, as shown above, referable to a considerable variety of types or species. In the experiments to be recorded it is shown that among the commoner types of *B. coli* (A sub-group) even after these organisms have been classified and separated into different species by cultural tests, it is still impossible to demonstrate any species specificity of an agglutinating anti-serum, and that agglutinating sera are specific only for the individual homologous strain.

Antisera were obtained to certain strains which represented common coliform types, (1) Type A1 (*B. MacConkey* No. 71), (2) Type A4 (*B. Grünthal*), (3) Type A3 (*B. vesiculosus*), (4) Type A2 (*B. coli communis*), and a number of other strains corresponding in all their characters with these types were tested with the immune sera.

Immune Sera.—Rabbits were immunised against the particular organisms by repeated intravenous injection of increasing amounts of bacillary emulsions sterilised at a temperature of 65° C. for half an hour. For this purpose twenty-four hours agar slope cultures were emulsified in convenient quantities of 0·85 per cent. sodium chloride solution. The series of doses were as follows: $\frac{1}{10}$, $\frac{1}{5}$, $\frac{1}{2}$, 1 and 2 emulsified agar slope cultures, given at intervals of 7 to 10 days. Ten days after the last injection the sera were tested with the strains used for immunisation, and if found of suitable value, *i. e.* agglutinating in a dilution of 1 : 2000 or in higher dilutions, the animal was bled and the serum after separation stored in sealed tubes. In the original experiments sterility of the serum was ensured by heating at 57° C. for one hour on three successive days. In some cases it was noted that there was a marked depreciation of the agglutinating value of the serum by heating, due apparently to the varying thermostability of the agglutinin. To obviate this the measures adopted for bleeding and collecting the serum were carried out with the utmost precautions to exclude contamination, and the serum was heated at 57° C. for only half an hour on two successive days.

Method of Carrying out the Agglutination Tests.—A twenty-four hours agar slope culture was emulsified in 5 c.c. of 0·85 per cent. salt solution, and the emulsion allowed to stand in the incubator for about one hour to allow the larger clumps and fragments of agar to deposit. The supernatant fluid was then decanted and made up to 10 c.c. Varying dilutions of the antiserum were mixed with equal volumes (0·5 c.c.) of bacillary emulsion, and the mixtures placed in narrow tubes in which the agglutination could be observed by the naked eye. As a control 0·5 c.c. of the bacillary emulsion was mixed with an equal volume of salt solution and included in the test series; this eliminated any fallacy due to auto-agglutination. It is to be noted, however,

that auto-agglutination was rarely seen among these bacilli. The tubes were placed in the incubator for one and a half hours, and at the expiry of that period, at room temperature for half an hour; they were again replaced in the incubator for two hours, when readings were taken of the results. Ultimately they were allowed to stand at room temperature till next day, when further readings, if necessary, were made. It was usually noted that the variations in temperature produced in this way set up convection currents in the fluid and this hastened agglutination. The degree of agglutination was determined by the amount of sediment in the various tubes as compared with the control or by the clarity or turbidity of the supernatant fluid as compared with the fluid in the control tube.

Complete agglutination is signified in the tables by + + + +, and lesser degrees by + + +, + +, and +.

Results Observed with Antisera to A Types 1, 2, 3 and 4.—Marked specificity for the individual strain on the part of these immune sera was observed. Tables VII, VIII, IX and X show that the only strains agglutinated to any extent by the *corresponding* antiserum were the particular strains used for immunisation. Thus, the antiserum to strain 1 type 1 agglutinated strain 1 in dilutions up to 1 : 50,000; 14 other strains of the same type were tested with the antiserum but none showed any agglutination by dilutions higher than 1 : 500, and 8 were not even agglutinated by a dilution of 1 : 100. A strain of type 3 also exhibited little reaction with the type 1 antiserum (Table VII). Similar results were obtained with antisera to types 2, 3 and 4.

While this restricted specificity was found to be the general rule with antisera to these common types of *B. coli* an exception has been noted: an antiserum to a type 2 (*B. coli communis*) was found to agglutinate a particular strain of type 1 (*B. MacConkey* No. 71) in a four times higher dilution than in the case of the homologous strain (Table XI), and this type 1 strain was not found to be specially susceptible to other *B. coli* agglutinins (Tables IX and X), *i. e.* it was not itself susceptible to other agglutinating sera nor did it show any tendency to auto-agglutination. Moreover, this agglutinin had no effect on a number of other strains of type 1 (Table XI).

To ascertain whether this peculiarity was a function of the immune animal, another antiserum to the same type 2 strain was obtained and the serum behaved in practically the same manner, indicating that this property of "paragglutination" was dependent on the particular strain (Table XI).

It is noteworthy that this instance of paragglutination is characterised by the more powerful effect of the paragglutinin than the primary agglutinin.

As is well known, the agglutinin has marked affinities for the homologous bacillus, and is absorbed or used up by the organisms during the process of agglutination. Moreover, a bacillary emulsion is capable of absorbing much

TABLE VII.—*Antiserum to Strain* 1, B. coli, *Sub-group A, Type* 1 (B. MacConkey *No.* 71).

Dilution	1 : 100	1 : 500	1 : 1000	1 : 5000	1 : 10,000	1 : 20,000	1 : 30,000	1 : 50,000
Strain 1, A1	++++	+++	+++	+++	+++	+++	++	+
Other strains having same characters, i. e. *of Type A*1.								
Strains 4, 6, 7, 10, 11, 12, 13, 15	0	0	0	0	0	0	0	0
Strains 3, 9	++	+	0	0	0	0	0	0
Strains 2, 8	+	0	0	0	0	0	0	0
Strains 5, 14	+++	++	0	0	0	0	0	0
Strain 1, A3	++	0	0	0	0	0	0	0

Controls showed no agglutination.

TABLE VIII.—*Antiserum to Strain* 1, B. coli, *Type A*2 (B. coli communis).

Dilution	1 : 200	1 : 400	1 : 800	1 : 1600	1 : 3200	1 : 6400	1 : 8000	1 : 10,000
Strain 1, A2	++++	++++	++++	++++	++++	+++	++	+
Other strains having same characters, i. e. *of Type A*2.								
Strains 2, 3, 4, 5, 6, 7, 8, 9, 10	0	0	0	0	0	0	0	0
Strain 11	+	0	0	0	0	0	0	0
Strain 1, A4	0	0	0	0	0	0	0	0

Controls showed no agglutination.

TABLE IX.—*Antiserum to Strain* 1, B. coli, *Type A*3 (B. vesiculosus)

Dilution	1 : 60	1 : 120	1 : 240	1 : 480	1 : 960	1 : 2000
Strain 1, A3	++++	++++	++++	+++	+++	++
Other strains having same characters, i. e. *of Type A*3.						
Strains 3, 4, 5, 6, 7	0	0	0	0	0	0
Strain 2	+	0	0	0	0	0
„ 8	++	+	0	0	0	0
„ 1, A1	+	0	0	0	0	0
„ 1, A4	+	0	0	0	0	0

Controls showed no agglutination.

TABLE X.—*Antiserum to Strain* 1, B. coli, *Type A*4 (B. Grünthal).

Dilution	1 : 50	1 : 100	1 : 200	1 : 400	1 : 800	1 : 1600	1 : 3200	1 : 8000	1 : 10,000
Strain 1, A4	++++	++++	++++	++++	++++	++++	++++	+++	+
Other strains having same characters, i. e. *of Type A*4.									
Strains 3, 4, 6, 8	0	0	0	0	0	0	0	0	0
„ 2, 5, 9	+	+	0	0	0	0	0	0	0
„ 7, 10	+	0	0	0	0	0	0	0	0
Strain 1, A1	+++	+	0	0	0	0	0	0	0
„ 1, A3	++	0	0	0	0	0	0	9	0

Controls showed no agglutination.

TABLE XI.—*First Antiserum to Strain* 1, B. coli, *Type A*2 (B. coli communis).

Dilution	1 : 100	1 : 1000	1 : 8000	1 : 10,000	1 : 20,000	1 : 30,000	1 : 40,000
Strain 1, A2	++++	++++	++	+	0	0	0
„ 1, A1 (*B. Mac-Conkey*, No. 71)	++++	+++	+++	+++	+++	+++	++

Ten other strains having same characters as Strain 1, A1 showed no agglutination with antiserum diluted 1 : 500.

Second Antiserum to Strain 1, *A*2.

Dilution	1 : 200	1 : 400	1 : 1000	1 : 2000	1 : 4000	1 : 8000	1 : 16,000
Strain 1, A2	++++	+++	+++	++	0	0	0
„ 1, A1	+++	+++	+++	+++	++	+	0

Controls showed no agglutination.

TABLE XII.—*Antiserum to Strain* 1, B. coli, *Type A*1 *absorbed at* 1 : 500 *by large Excess of Emulsion of*—

(1) Strain 1, A1 ;
(2) „ 2, A1 ;
(3) „ 1, A2.

Treated Sera.

Tested with Strain 1, A1 :	Dilution	1 : 600	1 : 1200	1 : 2400	1 : 4800	1 : 9600	1 : 12,000
	(1)	++	+	0	0	0	0
	(2)	++++	++++	++++	+++	+++	+
	(3)	++++	++++	++++	+++	+++	+

Controls showed no agglutination.

TABLE XIII.—*Antiserum to Strain* 1, *A*4 (B. Grünthal), *absorbed at* 1 : 1000 *by Excess of Emulsion of*—

(1) Strain 1, A4;
(2) „ 2, A4;
(3) „ 1, A3.

Treated Sera.

Tested with Strain 1, A4:	Dilution	1 : 1000	1 : 2000	1 : 4000	1 : 8000	1 : 10,000
	(1)	0	0	0	0	0
	(2)	++++	++++	++++	++	0
	(3)	++++	++++	++++	+	0

Controls showed no agglutination.

TABLE XIV.—(1) *Antiserum to Strain* 1, *A*2 (B. coli communis) *absorbed by Strain* 1, *A*1 (v. *Table* 11) *at* 1 : 1000 *dilution*; (2) *Same Antiserum absorbed by Strain* 1, *A*2 *at* 1 : 1000 *dilution.*

Treated Serum (1).

Dilution	1 : 1000	1 : 2000	1 : 3000	1 : 4000	1 : 6000	1 : 8000
Tested with:						
Strain 1, A2	++++	++++	++++	++++	+++	++
„ 1, A1	++	+	0	0	0	0

Treated Serum (2).

Tested with:						
Strain 1, A2	++	+	0	0	0	0
„ 1, A1	0	0	0	0	0	0

Controls showed no agglutination.

more agglutinin than is required for complete agglutination of the organisms. It was found that if these agglutinating sera were absorbed (1) by the homologous strain, (2) another strain of the same type, and (3) another strain of a different type, and then tested with the homologous strain, the agglutinin could be almost completely absorbed by the homologous strain, but that the absorptive effect of the other organisms was relatively weak and equal in degree (Tables XII and XIII).

For this purpose concentrated emulsions were prepared, and a certain dilution, 1 : 500 or 1 : 1000, of the antiserum was treated with the different organisms at 37° C. for two hours; the mixtures were centrifugalised till the supernatant fluids were quite clear; these were pipetted off and tested each with the homologous strain in various further dilutions.

Absorption tests were also carried out with the antiserum which showed the paragglutination phenomenon. It was found that while treatment of the serum by the strain used for immunisation removed the agglutinin both for this strain and the heterologous strain, the heterologous strain was only capable of absorbing its own agglutinin (Table XIV). This is true for coagglutination effects generally (Castellani), and showed that the two strains were not serologically identical.

It was thus quite impossible to establish any differentiation by agglutination tests between different types of these coliform bacilli, and no species specificity was displayed by their agglutinins; the specificity is restricted to the individual strain, and, by the ordinary agglutination reaction, and also by absorption tests, it is quite impossible to demonstrate that organisms of the same species (determined by cultural reactions) as the homologous strain, are more closely related to it than representatives of other types. Thus the individuality of the bacterial strain is most strikingly elicited. In this group strains are constantly assuming new characters (*v.* p. 360) and becoming highly specialised. *Hence we must assume that a high degree of individuality is attained by each strain and that differences of cultural characters within certain limits are of little significance.*

Results observed with antisera to B. types 1 *and* 2.—In the foregoing observations regarding the action of *B. coli* agglutinins, the commoner types, *B. MacConkey* No. 71, *B. coli communis, B. Grünthal, B. vesiculosus* belonging to the indol forming sub-group (A) of coliform bacilli were studied; further experiments were then carried out with agglutinating sera to certain of the B sub-group which are of less frequent occurrence.

Antisera were obtained to strains of types B1 (*B. MacConkey* No. 74) and B2 whose characters are shown in Table III. A number of other strains identical in their characters to these types were tested with the respective immune sera. The results with an antiserum to strain 1, type B1 are shown in Table XV. It was noted that while the strain used for immunisation was agglutinated by a 1 : 8000 dilution of the serum, three

other identical strains were only agglutinated by a lower dilution (1 : 100, 1 : 1000), and strains of sub-group A type 1 and B2 also were not agglutinated except by low dilutions. Strains 3 and 6 were agglutinated, however, to the same degree as the strain used for immunisation.

An antiserum to strain 3 was also obtained and tested with the other strains (including No. 1). The corresponding effect was found to occur, *i. e.* marked agglutination of strain 3 and also of strains 1 and 6, while the other strains were not affected to any marked extent (Table XVI). In this type therefore the specificity was not so restricted, and the homologous strain was not the only strain which showed marked agglutinability by the antiserum. Other strains of the same type were, however, not more agglutinable than a heterologous strain belonging to an entirely different sub-group. Thus no species differentiation could be elicited by means of these antisera.

In the case of the antiserum to a strain of B2, two other corresponding strains were also tested. The strain used for immunisation was agglutinated by dilutions up to 1 : 10,000 (Table XVII); strain 2 was agglutinated by dilutions up to 1 : 3200, but strain 3 showed a less degree of agglutinability (end-titre 1 : 800).

Among these B types there is a relative specificity of the agglutinin for the individual strain as in the case of the indol-forming types, but the results indicate that the specificity is much less restricted. In the case of agglutinating sera to A types 1, 2, 3 and 4, the strain used for immunisation showed marked agglutination, while other strains of the same types respectively were practically inagglutinable except by low dilutions of the serum. In the case of antisera to type B1 strains, other strains of the same type showed an almost equal agglutinability, and as regards the antiserum to strain 1, B2, of the two other corresponding strains, one was agglutinated by relatively high dilutions though not quite equal in agglutinability to the strain used for immunisation.

Results observed with antisera to B types 101 *and* 103 (*paracolon bacilli*). —Agglutinating sera for two types of the so-called paracolon bacilli, *i. e.* types which ferment glucose and mannite with gas production and do not ferment lactose or saccharose. In this case specificity for the individual strain was completely absent and exact species specificity was observed.

The immune serum to strain 1, B101, agglutinated this strain in dilutions as high as 1 in 8,000,000 (an unusually powerful agglutinin), and three other similar strains were agglutinated by equally high dilutions (Table XVIII).

It is of interest to note that strain 4, B101, underwent spontaneous variation in saccharose medium (fluid); so that a new strain was developed differing from the original strain in fermenting saccharose within twenty-four hours' growth (*v.* Table XXIX). Both the original and variant strain were equally agglutinable by the antiserum. The immune serum to strain 1,

B103, agglutinated three other similar strains to the same degree as the strain used for immunisation (Table XIX).

Thus among the coliform bacilli investigated serologically, different grades of specificity on the part of agglutinating sera have been noted.

(1) In the case of the commoner types, sub-group A, types 1, 2, 3 and 4 (gas producing, indol +, inosite −, lactose +), marked specificity for the individual strain was observed.

(2) In the case of certain less common types, sub-group B, types 1 and 2 (gas producing, indol −, inosite −, lactose +), absolute specificity for individual strains was not observed, but there was not complete specificity for the species or type as determined by cultural tests.

(3) In the case of certain paracolon types (B101 and 103) (gas producing, indol −, inosite −, lactose −) precise specificity for the cultural type was observed.

Complement Deviation Reactions.

As in the case of agglutination by immune sera to organisms of the A sub-group the specificity of the complement-deviating immune body is found to be related, not to the homologous species, but to the strain used for immunisation. This specificity for the individual strain was not so pronounced as in the case of the agglutinin and was only relative. While "group" agglutination among the different *B. coli* species was slight and often inappreciable, the complement-deviating antibody displayed marked "group" action within certain well-defined limits, and in the experiments to be recorded some indication of the biological relationships of different *B. coli* types has been elicited from a study of these group reactions.

Complement Deviation Methods.

Antigen: Emulsions of the bacilli in 0·85 per cent. salt solution were generally used as antigen; these were prepared by mixing an eighteen to twenty-four hours agar slope culture of the particular organism with a given quantity (10 c.c.) of salt solution. The whole agar surface had been inoculated abundantly so that a continuous growth was obtained, and by using tubes with agar surfaces of approximately equal size, the emulsions of different organisms generally exhibited an approximately equal degree of turbidity.* The emulsions were sterilised in a vaccine bath at 65° C. for half an hour; this is usually sufficient to ensure the killing of organisms of the *coli* group, and does not affect the antigenic value of the emulsions. These antigens generally exhibited a more or less degree of anti-comple-

* Table XX shows how closely the antigenic properties of these emulsions correspond; compare the deviation by the A4 antiserum + strains 2 A4, 1 A3 and 1 A1.

TABLE XV.—*Antiserum to Strain* 1, B. Coli, *Type B*1 (B. MacConkey, *No.* 74).

Dilution	1 : 100	1 : 500	1 : 1000	1 : 2000	1 : 4000	1 : 6000	1 : 8000	1 : 10,000
Strain 1, B1	++++	++++	++++	++++	++++	+++	++	0
,, 2, B1	++++	+++	++	0	0	0	0	0
,, 3, B1	++++	++++	++++	++++	++++	+++	++	0
,, 4, B1	++	0	0	0	0	0	0	0
,, 5, B1	+++	0	0	0	0	0	0	0
,, 6, B1	++++	++++	++++	++++	++++	+++	+	0
,, 1, A1	+++	++	0	0	0	0	0	0
,, 1, B2	+++	+	0	0	0	0	0	0

Controls showed no agglutination.

TABLE XVI.—*Antiserum to Strain* 3, B. coli, *Type B*1.

Dilution	1 : 50	1 : 100	1 : 800	1 : 1600	1 : 3200	1 : 6400
Strain 1, B1	++++	++++	++++	++++	+++	+
,, 2, B1	++++	+++	0	0	0	0
,, 3, B1	++++	++++	++++	++++	++	0
,, 4, B1	++++	+++	+	0	0	0
,, 5, B1	++	0	0	0	0	0
,, 6, B1	++++	++++	++++	+++	++	+
,, 1, A1	+++	+	0	0	0	0

Controls showed no agglutination.

TABLE XVII.—*Antiserum to Strain* 1, B. coli, *Type B*2.

Dilution	1 : 50	1 : 100	1 : 400	1 : 800	1 : 1600	1 : 3200	1 : 6400	1 : 10,000
Strain 1, B2	++++	++++	++++	++++	++++	++++	+++	+
,, 2, B2	++++	++++	++++	++++	+++	++	0	0
,, 3, B2	++++	++++	+++	++	0	0	0	0
,, 1, A1	+++	0	0	0	0	0	0	0

Controls showed no agglutination.

TABLE XVIII.—*Antiserum to Strain* 1, *B*101 (B. paracolon *type*).

Dilution	1 : 1,000,000	1 : 2,000,000	1 : 4,000,000	1 : 6,000,000	1 : 8,000,000	1 : 10,000,000
Strain 1, B101	++++	+++	++	++	+	0
,, 2, B101	++++	++++	+++	+++	++	0
,, 3, B101	++++	++++	++++	+++	++	0
,, 4, B101	+++	++++	++++	+++	++	0
,, 1, A1	0	0	0	0	0	0
,, 1, B1	0	0	0	0	0	0
,, 1, B103	+++	0	0	0	0	0

Note the very high degree of potency attained by this particular agglutinating serum.

TABLE XIX.—*Antiserum to Strain* 1, *B*103 (B. paracolon *type*).

Dilution	1 : 500	1 : 1000	1 : 2000	1 : 4000	1 : 8000	1 : 16,000	1 : 32,000
Strain 1, B103	++++	++++	++++	++++	+++	+++	+
,, 2, B103	++++	++++	++++	++++	+++	+++	+
,, 3, B103	++++	++++	++++	++++	+++	++	+
,, 4, B103	++++	++++	++++	++++	+++	++	++
,, 2, B101	++	0	0	0	0	0	0

Controls showed no agglutination.

mentary action by themselves; this effect appeared to depend mainly on two factors—(1) the particular specimen of complement, and (2) the presence in the emulsion of fragments of agar, which, after heating of the emulsion, render it extremely viscous. It was generally found better to employ complement-serum eighteen to twenty-four hours after its withdrawal as fresh complement tends to be more deviable by anti-complementary agents.*

TABLE XX.—*Lysis of 0·5 c.c. 5 per cent. Ox Blood + 5 Doses Immune Body.*

Antiserum to Strain 1, A4 (*B. Grünthal*).	Bacillary emulsion.	Doses of complement.					Complement deviated by emulsion alone.	
		2 D.	5 D.	10 D.	15 D.	20 D.	2 D.	5 D.
0·025 c.c.	0·4 c.c. Strain 1, A4	0	0	0	0	0	Trace	Just c.
0·01 „		0	0	0	0	0	—	—
0·005 „		0	0	0	0	0	—	—
0·001 „		0	0	0	0	Com.	—	—
0·0005 „		0	0	Al. com.	Com.	—	—	—
0·0001 „		0	Dist.	Com.	—	—	—	—
0·025 „	0·4 c.c. Strain 2, A4	0	0	0	0	0	Just c.	Com.
0·01 „		Dist.	Mkd.	Al. com.	Com.	—	—	—
0·05 „		Mkd.	Al. com.	Com	—	—	—	—
0·001 „		Al. com.	Com.	—	—	—	—	—
0·025 „	0·4 c.c. Strain 1, A3	0	0	0	0	0	V. mkd.	Com.
0·01 „		Dist.	Dist.	Mkd.	Com.	—	—	—
0·005 „		Dist.	Mkd.	Com.	—	—	—	—
0·001 „		Mkd.	Just c.	—	—	—	—	—
0·025 „	0·4 c.c. Strain 1, A1	0	0	0	0	0	Mkd.	Al. c.
0·01 „		0	Dist. trace	V. mkd.	Com.	—	—	—
0·005 „		0	Dist. trace	Al. com.	—	—	—	—
0·001 „		Trace	Mkd.	Com.	—	—	—	—

0·025 c.c. antiserum alone deviated 2 D. of complement.
0·01 „ „ „ „ no complement.

In this and in subsequent tables: C. or Com. = complete lysis. Al. c. or Al. Com. = almost complete lysis. V. mk. or V. mkd. = very marked lysis. Mk. or Mkd. = marked lysis. Dt. or Dist. = distinct lysis. Tr. or Trace = trace of lysis. F. Trace = faint trace. 0 = no lysis.

Apart from this, individual animals yield complements which may display varying degrees of deviability; some are extremely deviable, while others are little affected by the usual inhibitory agents, and it is thus impossible to predicate how a certain specimen of complement-serum will behave. As regards the other complicating factor, it was found essential before heating to centrifugalise the emulsions for one or two minutes. This deposited any agar fragments and the supernatant emulsion was then pipetted off.

* This is well known as regards the Wassermann reaction.

It was thus possible to obtain bacillary emulsions which, with suitable complements, showed little anti-complementary effect in the quantities used.

Bacillary extracts have been extensively used in place of simple emulsions. These were originally employed by Wassermann and Bruck, and various workers have preferred them on the ground that they are less anti-complementary than emulsions. I have prepared extracts by Dean's method of alternately freezing and thawing emulsions and then removing the

TABLE XXI.—*Lysis of* 0·5 *c.c.* 5 *per cent. Suspension Ox Blood* + 5 *Doses Immune Body.*

Antiserum to Strain 1, A1 (*B. No.* 71 *McC.*).	Bacillary emulsion 0·4 c.c.	Doses of complement.					Complement deviated by emulsion alone.	
		2 D.	4 D.	7 D.	12 D.	20 D.	2 D.	4 D.
0·01 c.c.	Strain 1, A1 (B. No. 71)	0	0	0	0	0	—	—
0·005 ,,		0	0	0	0	0	—	—
0·001 ,,		0	0	0	0	0	Mkd.	Just c.
0·0005 ,,		0	0	0	0	0	—	—
0·0001 ,,		0	Dist.	Just c.	—	—	—	—
0·01 ,,	Strain 2, A1	0	0	0	0	0	—	—
0·005 ,,		0	0	0	Trace	Com.	Mkd.	Just c.
0·001 ,,		0	Dist.	Mkd.	Al. com.	Com.	—	—
0·01 ,,	Strain 1, A6 (*B. neapolitanus*)	0	0	0	0	Trace	—	—
0·005 ,,		0	0	0	Trace	V. mkd.	V. mkd.	Just c.
0·001 ,,		0	Mkd.	Mkd.	V. mkd.	Com.	—	—
0·01 ,,	Strain 1, A2 (*B. coli communis*)	0	0	0	0	Trace	—	—
0·005 ,,		0	0	0	Trace	Mkd.	Mkd.	Just c.
0·001 ,,		0	Trace	Mkd.	V. mkd.	Com.	—	—
0·01 ,,	Strain 1, A4 (*B. Grünthal*)	0	0	Dist.	Mkd.	Al. com.	Com.	Com.
0·005 ,,		0	Dist.	Mkd.	V. mkd.	Al. com.	—	—
0·001 ,,		Dist.	V. mkd.	Al. com.	Com.	Com.	—	—

0·025 c.c. antiserum alone deviated 2 D. of complement.
0·01 ,, ,, ,, ,, no complement.

bacteria by centrifugalisation. These extracts were found no less inhibitory in certain amounts with various specimens of complement than the ordinary emulsions. Throughout the experiments emulsions have been used as antigen; they are easily prepared, and with suitable complements exhibit little inhibitory effect.

The *immune sera* used were those already referred to in connection with the agglutination experiments.

Guinea pig's serum was used as *complement*, and ox-blood corpuscles sensitised with five doses of a haemolytic immune body (from the rabbit) was employed as the *haemolytic system*.

The method of carrying out the tests, where careful comparisons were made of the deviation of an antiserum along with different bacillary strains, was as follows: varying quantities of the serum were added to a fixed quantity of the antigen, and then a quantitative estimation of the amount of complement deviated by these mixtures was made by adding varying amounts of complement from 3 M.H.D up to 20 M.H.D. (for 0·5 c.c. of the test blood suspension), incubating the mixtures for $1\frac{1}{4}$ hours at 37° C., and then

TABLE XXII.—*Lysis of* 0·5 *c.c.* 5 *per cent. Suspension Ox Blood* + 5 *Doses Immune Body.*

Antiserum to Strain 1, A3 (*B. vesiculosus*).	Bacillary emulsion 0·4 c.c.	Doses of complement.					Complement deviated by emulsion alone.		
		2 D.	5 D.	10 D.	15 D.	20 D.	2 D.	5 D.	7 D.
0·025 c.c.	Strain 1, A3 (*B. vesiculosus*)	0	0	0	0	0	—	—	—
0·01 „		0	0	0	0	0	—	—	—
0·005 „		0	0	0	0	0	V. mk.	Al. com.	Com.
0·001 „		0	0	0	0	0	—	—	—
0·0005 „		0	0	Mkd.	Al. com.	Com.	—	—	—
0·0001 „		Dist.	Mkd.	Com.	—	—	—	—	—
0·025 „	Strain 2, A3	0	0	0	0	Dist.	—	—	—
0·01 „		0	0	Dist.	V. mkd.	Com.	Dist.	Al. com.	Com.
0·005 „		0	Trace	Mkd.	Com.	Com.	—	—	—
0·001 „		Trace	Mkd.	Com.	Com.	Com.	—	—	—
0·025 „	Strain 1, A4 (*B. Grün-thal*)	0	0	0	F. tr.	Dist.	—	—	—
0·01 „		0	0	Dist.	Al. com.	Com.	Dist.	Al. com.	Com.
0·005 „		0	Trace	V. mkd.	Com.	Com.	—	—	—
0·001 „		Trace	Dist.	Com.	Com.	Com.	—	—	—
0·025 „	Strain 1, A9 (B. No. 106 *McConkey*)	0	0	0	F. tr.	Mkd.	—	—	—
0·01 „		0	0	Trace	V. mkd.	Com.	Dist.	Al. com.	Com.
0·005 „		0	0	Dist.	Com.	Com.	—	—	—
0·001 „		Trace	Dist.	Com.	Com.	Com.	—	—	—

0·025 c.c. antiserum alone deviated 1 D. of complement.
0·01 „ „ „ „ no complement.

adding 0·5 c.c. of the blood suspension. After a further hour's incubation the results were read.

Control tests were also carried out to determine the number of doses of complement absorbed by the antigen and immune serum respectively. At the same time the dose of complement after incubation for $1\frac{1}{2}$ hours was ascertained by adding suitable amounts of the complement-serum to tubes containing a volume of salt solution equal to that of the antigen used in the tests, incubating along with the other tubes and then adding the test corpuscles.

The number of doses of complement deviated was taken as one less than

that represented by the smallest amount in the series which produced complete lysis (complete lysis occurring when one dose is left free). The number of doses deviated by the serum and emulsion separately was deducted from the number of doses absorbed by the serum and antigen in combination, and the result represented the exact degree of deviation produced apart from the inhibitory effects of serum and bacillary emulsion. Thus any inequality in the anti-complementary action of different antigens was allowed for.

By this method, in which the amount of complement deviated by varying amounts of antiserum along with a fixed quantity of bacillary antigen is

TABLE XXIII.—*Lysis of 0·5 c.c. 5 per cent. Suspension Ox Blood + 5 Doses Immune Body.*

Bacillary emulsion.	Antiserum to Strain 1, A1 (*B. No. 71 McC.*).	Doses of complement.					Complement deviated by emulsion alone.	
	0·025 c.c.	2 D.	4 D.	7 D.	12 D.	20 D.	2 D.	4 D.
Strain 1, A1								
0·2 c.c.	—	0	0	0	0	F. tr.	Com.	Com.
0·12 „	—	0	0	F. tr.	F. tr.	Dist.	Com.	Com.
0·04 „	—	0	Trace	Dist.	Dist.	Mkd.	Com.	Com.
0·02 „	—	0	Dist.	Mkd.	Mkd.	Com.	Com.	Com.
Strain 1, A2								
0·2 c.c.	—	0	0	0	0	0	Com.	Com.
0·12 „	—	0	0	0	Trace	V. mkd.	Com.	Com.
0·04 „	—	0	F. tr.	Mkd.	Com.	Com.	Com.	Com.
0·02 „	—	Trace	Dist.	V. mkd.	Com.	Com.	Com.	Com.
Strain 2, A1								
0·2 c.c.	—	0	0	0	0	0	Com.	Com.
0·12 „	—	0	0	0	Mkd.	Mkd.	Com.	Com.
0·04 „	—	0	F. tr.	Trace	Mkd.	Com.	Com.	Com.
0·02 „	—	0	Trace	Al. com.	Com.	Com.	Com.	Com.

0·025 c.c. antiserum alone deviated 1 D. of complement.

tested, it is possible to precisely differentiate *B. typhosus* from *B. paratyphosus* A and B. In one of my own experiments 0·0002 c.c. of an anti-typhoid serum along with a strain of *B. typhosus* (not that used for immunisation) deviated over seventeen doses of complement, while with *B. paratyphosus* A 0·025 c.c. of the serum was required to produce a deviation of ten doses and 0·0002 c.c. produced practically no deviation. Thus by careful quantitative comparisons it is possible to establish a precise species differentiation between certain allied organisms. In the case of *B. typhosus* the relative specificity is for the species.

Results observed with antisera to A types 4, 1 *and* 3.—In the case of the antiserum to strain 1, A4 (*B. Grünthal*), over fifteen doses of complement were absorbed by the combination of 0·001 c.c. of antiserum and the strain

used for immunisation, while with another strain of the same type (No. 2) it was necessary to employ 0·025 c.c. of antiserum to obtain this degree of complement absorption, and on diminishing the amount of antiserum there was a rapid falling off in the amount of complement deviated (Table XX). With representatives of other typical *B. coli* varieties, the amounts of complement deviated by different quantities of antiserum were practically the same as those deviated with the No. 2 strain of type B4. Thus even by varying the amount of antiserum no demarcation could be demonstrated in the A sub-group between, for example, types 1 and 3 on the one hand and type 4 on the other. This was found to be true also in the case of antisera to types 1 and 3 of sub-group A (Tables XXI and XXII).

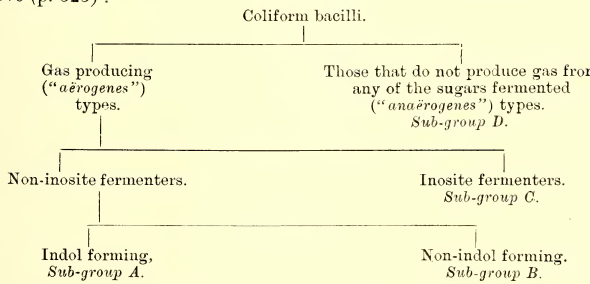
Experiments were also carried out in which the amount of antiserum was maintained constant and the quantity of antigen varied; with the antiserum to strain 1 of type 1 no differentiation could be established between another type 1 strain and a strain of a different type (type 2) (Table XXIII).

Group Reactions.

The group reaction was then studied to ascertain its significance as regards the biological relationships between different types and the classification or grouping of these organisms.

Group action by complement deviating sera has been studied in other bacterial classes; thus Sacquépée found that the food-poisoning group could be classified into two sub-groups (Enteritis I and II). An immune serum to an organism of sub-group I reacted with other sub-group I types but not with organisms of sub-group II; similarly an antiserum to a sub-group II type reacted only with other organisms of the same sub-group.

On testing the antiserum to strain 1 type 4 (*B. Grünthal*) along with a large number of different coliform strains (typical and atypical) it was found that the group reaction was limited to a certain class. If these coliform bacilli are divided up as follows, as in the original classification given above (p. 323):



then sub-group A will comprise all the typical varieties, *e.g. B. MacConkey*

TABLE XXIV.—*Lysis of 0·5 c.c. 5 per cent. Suspension Ox Blood + 5 Doses Immune Body.*

ANTISERUM TO STRAIN 1, A4 (B. GRÜNTHAL), 0·025 c.c.

Different strains of type		1	1	2	1	1	2	2	3	4	3	5	4	5	6	7	6	30	11	10	2	3	9	101	34	19	19	15	12		22	9	1	
Bacillary emulsion 0·4 c.c.	Sub-group	A	C	C	D	B	B	A	B	B	A	B	A	A	A	A	B	A	A	B	D	C	B	B	A	A	A	A	A		A	A	A	
					Coli anærogenes																*Coli anærogenes*			Paracolon						*B. para-typhosus* B. Conjunctivæ			*B. proteus*
Doses of complement	3D	O	J. C.	V. mk.	Al. C.	Mk.	O	O	Al. C.	Dt.	O	J. C.	O	O	O	O	O	O	O	Al. C.	O	C	C	C	O	O	O	O	O	C	Mk.	Tr.	C	C
	5D	O	C	C	C	Al. C.	Mk.	O	C	Mk.	O	C	O	O	O	O	Tr.	O	O	C	Tr.	C	C	C	O	O	O	O	O	C	Al. C.	Dt.	C	C
	10D	O	C	C	C	C	C	O	C	C	O	C	O	O	O	O	Mk.	O	O	C	C	C	C	C	O	O	O	O	O	C	C	Al. C.	C	C
	15D	O	C	C	C	C	C	O	C	C	O	C	O	O	O	O	C	O	O	C	C	C	C	C	O	O	O	O	O	C	C	C	C	C
	20D	O	C	C	C	C	C	Tr.	C	C	Tr.	C	O	Tr.	O	O	C	Tr.	Tr.	C	C	C	C	C	O	O	O	O	O	C	C	C	C	C

ANTISERUM TO STRAIN 1, A3 (B. VESICULOSUS), 0·025 c.c.

Doses of complement	3D		Dt.	V. mk.	Dt.	Dt.			Tr.		O	J. C.	O	O	O	O	O												Tr.	C	Mk.	Tr.	C	
	5D		Al. C.	C	V. mk.	V. mk.			Dt.		O	J. C.	O	O	O	O	Tr.												Tr.	C	C	Dt.	C	
	10D		C	C	C	C			C		O	J. C.	O	O	O	O	Mk.												Tr.	C	C	Al. C.	C	
	15D		C	C	C	C			C		O	J. C.	O	Tr.	O	O	C												Mk.	C	C	C	C	
	20D		C	C	C	C			C		Tr.	J. C.	Mk.	Dt.	F. tr.	O	C												V. mk.	C	C	C	C	
Complement deviated by emulsion alone		8D	2D	3D	2D	4D	5D	4D	5D	4D	3D	2D	4D	5D	4D 6D	3D	7D	3D	4D	4D	6D	2D	2D	2D	4D	4D	6D	4D	4D	3D	4D	4D	3D	4D

No. 71, *B. coli communis*, etc. It was found that the group effect with the A4 antiserum was limited to these types, *i.e.* to sub-group A ; for example with representatives of this sub-group over fifteen doses of complement were

TABLE XXV.

Antiserum to Strain 1, A4 (*B. Grünthal*).	Bacillary emulsion.	Doses of complement.					Complement deviated by emulsion alone.		
		2 D.	5 D.	10 D.	15 D.	20 D.	2 D.	5 D.	7 D.
0·025 c.c.	0·4 c.c.	0	0	0	0	0	Mkd.	Com.	—
0·01 „	Strain 2, A4,	0	0	Trace	Dist.	Al. com.	—	—	—
0·005 „	lactose +,	0	0	V. mkd.	Com.	—	—	—	—
0·001 „	indol +	Trace	Dist.	Al. Com.	—	—	—	—	—
0·025 „	0·4 c.c. Strain A12,	0	0	0	0	0	Mkd.	Com.	—
0·01 „	lactose —,	0	0	0	Dist.	V. mkd.	—	—	—
0·005 „	adonite —,	0	Trace	Al. com.	Com.	—	—	—	—
0·001 „	inosite —, indol +	Trace	Dist.	Al. com.	—	—	—	—	—
0·025 „	0·4 c.c. Strain A19, lactose +	0	0	0	0	0	—	Mkd.	Com.
0·01 „	after mu-	0	0	0	Trace	Mkd.	—	—	—
0·005 „	tation,	0	0	Dist.	Mkd.	Com.	—	—	—
0·001 „	adonite —, inosite —, indol +	0	Dist.	V. mkd.	Com.	—	—	—	—
0·025 „	0·4 c.c. Strain A15,	0	0	0	0	0	Mkd.	Com.	—
0·01 „	lactose +,	0	0	0	0	Trace	—	—	—
0·005 „	adonite +,	0	0	Dist.	Com.	—	—	—	—
0·001 „	inosite —, indol +	0	Trace	Com.	—	—	—	—	—
0·025 „	0·4 c.c. Strain A34,	0	0	0	0	0	Mkd.	Com.	—
0·01 „	lactose —,	0	0	0	Trace	Com.	—	—	—
0·005 „	indol +,	0	Dist.	V. mkd.	Al. com.	Com.	—	—	—
0·001 „	inosite —	Trace	Dist.	Com.	Com.	Com.	—	—	—
0·025 „	0·4 c.c. Strain A6,	0	0	0	0	0	—	—	—
0·01 „	lactose +,	0	0	0	0	0	Dist.	Al. com.	Com.
0·005 „	indol +,	0	0	Trace	Al. com.	Com.	—	—	—
0·001 „	inosite —	0	Dist.	Com.	Com.	Com.	—	—	—

deviated in the presence of 0·025 c.c. of the antiserum, while with representatives of the other sub-groups B, C and D, in the presence of the same amount of antiserum, not more than five doses were deviated, and with some no deviation was observed (Table XXIV). Some of the strains were tested both with the A4 antiserum and also with the A3 antiserum and the results

were found to correspond (Table XXIV). It is noteworthy that certain representatives of sub-group A whose fermentative reactions did not correspond to those of the accepted *B. coli* types deviated in the presence of 0·025 c.c. of antiserum over 15 doses of complement, and even by varying the amount of antiserum one could not differentiate them from an A4 strain other than strain 1, *i. e.* the strain used for immunisation (Table XXV). Included among these were two strains which according to the usual criteria would not have been classed as typical *B. coli*, viz. two non-lactose-fermenters, and a

TABLE XXVI.—*Lysis of* 0·5 *c.c.* 5 *per cent. Ox Blood Suspension* + 5 *Doses immune body.*

Antiserum to Strain 3, B1 (*B. Mac-Conkey* No. 74).	Bacillary emulsion 0·4 c.c.	Doses of complement.					Complement deviated by emulsion alone.	
		3 D.	5 D.	10 D.	15 D.	20 D.	3 D.	5 D.
0·005 c.c.	Strain 3, B (*B. No.* 74 *MacConkey*)	0	0	0	0	0	—	—
0·001 ,,		0	0	Dist.	Al. com.	Com.	Mk.	Com.
0·0005 ,,		0	Dist.	Mk.	Com.	Com.	—	—
0·0001 ,,		Trace	V. mk.	Al. com.	Com.	Com.	—	—
0·025 ,,	Strain 2, B1 (*B. No.* 74 *MacConkey*)	0	0	0	0	0	—	—
0·01 ,,		0	0	0	Trace	Al. com.	Mk.	Com.
0·005 ,,		0	0	Trace	Al. com.	Com.	—	—
0·005 ,,	Strain 1, B2	0	0	0	Trace	Dist.	—	—
0·001 ,,		0	Trace	Dist.	Mk.	V. mk.	V. mk.	Just c.
0·0005 ,,		0	Trace	Mk.	Al com.	Com.	—	—
0·0001 ,,		Trace	Mk.	V. mk.	Just c.	Com.	—	—
0·005 ,,	Strain 2, B2	0	0	0	0	Trace	—	—
0·001 ,,		0	Trace	Dist.	Mk.	Al. com.	Mk.	Com.
0·0005 ,,		0	Trace	Mk.	Just c.	Com.	—	—
0·0001 ,,		Trace	Mk.	Al. com.	Just c.	Com.	—	—

0·025 c.c. antiserum alone deviated 1 D. of complement.

strain which only fermented lactose after mutation; these strains all produced indol. *The fact that such Gram-negative bacilli behave in complement-deviation experiments with an antiserum to a strain of typical* B. coli *similarly to other typical organisms strongly suggests that lactose fermentation, which has always been considered one of the most important characters of* B. coli, *can hardly be taken as of more importance biologically than other sugar reactions.*

Among the 31 strains of sub-groups A, B, C and D examined in this way, three A types (Nos. 1, 9 and 22) did not react characteristically with the A4 and A3 antisera, *i. e.* the group reaction was absent in the case of the No. 1 and 22 strains and not well marked in the case of the No. 9 strain (Table XXIV). The No. 22 strain was isolated from a case of conjunctivitis in which it was present in pure culture; the Nos. 1 and 9 strains were of direct faecal

origin. On the other hand no representatives of the other sub-groups B, C and D exhibited any reaction with the A4 and A3 antisera.

TABLE XXVII.—*Lysis of* 0·5 *c.c.* 5 *per cent. Ox Blood Suspension* + 5 *Doses Immune Body.*

Antiserum to Strain 3, B1 (*B. No.* 74 *MacConkey*).	Bacillary emulsion 0·4 c.c.	Doses of complement.					Complement deviated by emulsion alone.	
		3 D.	5 D.	10 D.	15 D.	20 D.	3 D.	5 D.
0·05 c.c.	Strain 2, B1 (*B. No.* 74 *MacConkey*)	0	0	0	0	Trace	—	—
0·025 ,,		0	0	F. tr.	Dist.	Mk.	Mk.	Com.
0·01 ,,		0	0	Dist.	V. mk.	Com.	—	—
0·005 ,,		0	Trace	Mk.	Com.	Com.	—	—
0·05 ,,	Strain of B3	0	0	0	0	Dist.	—	—
0·025 ,,		0	0	Trace	Dist.	Mk.	V. mk.	Com.
0·01 ,,		0	Trace	Dist.	Mk.	Com.	—	—
0·005 ,,		0	Dist.	V. mk.	Com.	Com.	—	—
0·05 ,,	Strain of B10	0	0	0	0	0	—	—
0·025 ,,		0	0	0	Trace	Dist.	Dist.	Just c.
0·01 ,,		0	0	Trace	Dist.	Al. com.	—	—
0·005 ,,		0	Trace	Dist.	Al. com.	Com.	—	—
0·05 ,,	Strain of B10	0	0	0	0	0	—	—
0·025 ,,		0	0	0	0	Dist.	Dist.	Just c.
0·01 ,,		0	0	Dist.	Mk.	V. mk.	—	—
0·005 ,,		0	Trace	Dist.	V. mk.	Com.	—	—
0·05 ,,	Strain of B9	0	0	0	0	0	Mk.	Com.
0·05 ,,	Strain of B4	0	0	0	0	0	Mk.	Com.
0·05 ,,	Strain A1, (*B. No.* 71 *MacConkey*)	Mk.	Com.	Com.	Com.	Com.	Mk.	Com.
0·05 ,,	Strain of A6 (*neapolitanus*)	V. mk.	Com.	Com.	Com.	Com.	V. mk.	Com.
0·05 ,,	Strain A3 (*B. coli communis*)	Al. com.	Com.	Com.	Com.	Com.	Al. com.	Com.
0·05 ,,	Strain of C1	Mk.	Com.	Com.	Com.	Com.	Mk.	Just c.
0·05 ,,	Strain of C2	Mk.	Com.	Com.	Com.	Com.	Mk.	Com.
0·05 ,,	Strain of *D6 Coli anaërogenes.*	Mk.	Com.	Com.	Com.	Com.	Mk.	Com.
0·05 ,,	*B. paratyphosus* B	Dist.	Mk.	Com.	Com.	Com.	Mk.	Com.
0·025 ,,		Mk.	Al. com.	Com.	Com.	Com.	—	—
0·01 ,,		V. mk.	Com.	—	—	—	—	—

TABLE XXVII (*continued*).

Antiserum to Strain 3, B1 (*B. No. 74 MacConkey*).	Bacillary emulsion 0·4 c.c.	Doses of complement.					Complement deviated by emulsion alone.	
		3 D.	5 D.	10 D.	15 D.	20 D.	3 D.	5 D.
0·05 c.c.	Strain of paracolon bacillus, lactose —, indol —, inosite —, glucose +, B 101	Dist.	Mk.	Al. com.	Com.	Com.	—	—
0·025 „		Dist.	V. mk.	Com.	Com.	Com.	—	Just c.
0·01 „		Mk.	V. mk.	Com.	Com.	Com.	—	—
0·005 „		Mk.	Com.	Com.	Com.	Com.	—	—
0·05 „	Strain of paracolon bacillus, B103	Trace	Dist.	Al. com.	Com.	Com.	—	—
0·025 „		Dist.	Mk.	Just c.	Com.	Com.	—	Com.
0·01 „		Dist.	V. mk.	Com.	Com.	Com.	—	—
0·005 „		Dist.	Com.	Com.	Com.	Com.	—	—
0·05 „	Strain of *B. coli mutabilis* B103 before mutation	Mk.	Al. com.	Com.	Com.	Com.	—	—
0·025 „		V. mk.	Com.	Com.	Com.	Com.	Just c.	Com.
0·01 „		V. mk.	Com.	Com.	Com.	Com.	—	—
0·005 „		Just c.	Com.	Com.	Com.	Com.	—	—
0·05 „	Strain of, B105, lactose — (+ after mutation), inosite —, indol —	Mk.	Com.	Com.	Com.	Com.	Just c.	Com.
0·05 „	Strain of B102, lactose — (+ after mutation), inosite —, indol —,	V. mk.	Com.	Com.	Com.	Com.	Just c.	Com.
0·05 „	*B. proteus*	Mk.	Com.	Com.	Com.	Com.	Just c.	Com.

0·05 c.c. of antiserum alone deviated 1 D. of complement.

Results observed with an antiserum to a B. type No. 1.—The specificity and group action of an antiserum to a strain belonging to one of the common types of sub-group B was also investigated. The antiserum to strain 3, B1 (*B. MacConkey* No. 74), whose agglutinating properties have already been referred to, was employed for this purpose.

It was found that the serum displayed no specificity either for the individual strain or the type to which it belonged. In fact certain strains of other different types within the sub-group B exhibited as much affinity (as determined by complement-deviation tests) for the serum as the strain

used for immunisation (Table XXVI). The group reaction was strictly limited to types belonging to the B sub-group. Thus with a number of strains of sub-group A, C and D practically no deviation was obtained (Table XXVII).

In the case of sub-group A it was found that by deviation tests non-lactose-fermenters and organisms which only fermented lactose after mutation could be classed along with the typical lactose-fermenting types. In sub-group B non-lactose-fermenters (*B. paracolon* types) and strains which developed lactose-fermenting mutants (*B. coli mutabilis* types) could not be identified with the lactose-fermenting types, *i.e.* the group action of an anti-serum to a lactose-fermenter was limited to those types which fermented lactose in primary culture.

As a result of these serological findings some indication has been elicited of the biological grouping of the different cultural types of coliform bacilli.

The results may be summarised as follows:

In sub-group A (gas-forming, indol +, inosite −, gelatin −) as determined by tests with antisera to types 1, 2, 3 and 4, there is a high degree of specificity of the agglutinin for the individual strain used for immunisation, but no evidence of specificity for the type, or group action towards other organisms of the sub-group; there is a relative specificity of the complement-deviating immune body, as determined with antisera to types 1, 3 and 4, for the individual strain but not for the cultural type; there is, however, a well-marked group reaction limited to strains of the sub-group irrespective of other cultural reactions (*e.g.* lactose, dulcite, saccharose, inulin fermentation, motility), and not extending to the B, C or D sub-groups, *B. paratyphosus B*, or *B. proteus*.

In sub-group B, as determined by observations with antisera to types 1 and 2, there is a more limited degree of specificity of agglutinating antisera for the individual strain but not complete specificity for the cultural type; there is absence of relative specificity of the complement-deviating antibody (antiserum to type I) for the individual strain, and the group reaction, as far as my observations go in the case of the types tested, is limited to the lactose-fermenting types, and does not extend to the A, C or D sub-groups, the non-lactose-fermenting types, of the B sub-group, the types which only fermented lactose after mutation, *B. paratyphosus B* or *B. proteus*.

While among the indol +, inosite −, gas + types lactose-fermentation appeared of no more significance than certain other reactions, in the case of the indol − inosite −, gas + types the lactose-fermenting types seemed to be separately grouped as apart from the non-lactose-fermenters and those which only fermented lactose after mutation.

Also in the case of two types of non-lactose-fermenters of the B sub-group exact specificity of the agglutinin for the type or species was noted,

and this also differentiated these organisms from the lactose-fermenting types.

The Comparative Resistance to Brilliant Green of Different Types of Coliform Bacilli with Reference to the Classification of these Organisms.

In the course of certain observations on the enrichment of *B. typhosus* by culture from faeces in fluid media containing brilliant green (Browning, Gilmour and Mackie), it was noted that different types of *B. coli* exhibited different degrees of susceptibility to this chemical. The "typical" varieties (sub-group A, types 1, 2, 3, etc.) were completely inhibited in their growth on culture medium by concentrations of brilliant green which had no effect on *B. typhosus*, but it was noted that the inosite fermenters (sub-group C) on the contrary exhibited a resistance to the dye greater even than that of the typhoid bacillus.

As this appeared to be a striking difference between two *B. coli* sub-groups already classified separately on an entirely different basis, there seemed some likelihood that the study of the behaviour of different coliform types towards this dye might throw some further light on the biological relationships of the various cultural types.

For this purpose a series of representative strains from the four sub-groups were tested as regards the inhibition of their growth in peptone-water-agar by different quantities of brilliant green incorporated in the medium.

The concentrations of the dye tested were 0·16, 0·22, 0·32, 0·42 c.c. of a 1:10,000 watery solution in 10 c.c. of the medium.

Emulsions of the various organisms were made in sterile salt solution, of such density that the fluid showed a mere trace of turbidity to the eye, and cultures were made by taking one loopful of the emulsion and spreading it on the medium in stroke form (one stroke only). On ordinary medium this inoculation produced an abundant line of growth along the needle tract. One plate was, of course, used to accommodate several strokes from different organisms.

The plates were incubated for forty-eight hours and readings were made after twenty-four and forty-eight hours.

With two exceptions all the sub-group A organisms tested proved relatively susceptible (as compared with *B. typhosus*) to the dye, including non-lactose-fermenters (Table XXVIII); the exceptions were (1) a strain of type 9 and (2) a strain of type 22 (isolated from a case of conjunctivitis); another strain of type 9, however, corresponded in its behaviour to the other A types. It is noteworthy that these two strains also differed in the complement-deviation experiments from other A types.

TABLE XXVIII.—*Brilliant Green* 1 : 10,000 *watery solution per* 10 *c.c. peptone-water-agar. Observations after* 24 *hours' incubation.*

Strains of	1 0·16 c.c.	2 0·22 c.c.	3 0·32 c.c.	4 0·42 c.c.
Sub-group A.				
Type 1 (*B. MacConkey*, No. 71)	++	—	—	—
,, 3 (*B. vesiculosus*)	+	+	—	—
,, 3	++	—	—	—
,, 5 (*B. Schafferi*)	+	—	—	—
,, 1	++	—	—	—
,, 6 (*Neapolitanus*)	+	—	—	—
,, 4 (*B. Grünthal*)	+	—	—	—
,, 3	+	+	—	—
,, 6	+	—	—	—
,, 15	+	—	—	—
,, 30	+	—	—	—
,, 2	++	—	—	—
,, 35 (*Lactose* —)	—	—	—	—
,, 2	+	—	—	—
,, 9	+	+	—	—
,, 13	—	—	—	—
,, 28	+	—	—	—
,, 33	+	—	—	—
,, 12	++	—	—	—
,, 9	+++	+++	++	—
,, 2	++	—	—	—
,, 4	+	—	—	—
,, 3	+	—	—	—
,, 1	++	—	—	—
,, 22	++++	+++	++	—
B. typhosus	++++	++++	++	—
B. proteus	+	—	—	—
Sub-group B.				
Type 1	++++	++++	++	++
,, 9	++++	++++	++	+
,, 10	++++	++++	++	++
,, 2	++++	++++	++	—
,, 2	+++	+++	++	—
,, 10	+++	+++	+	—
,, 1	++++	+++	++	+
,, 101 (*paracolon*)	—	—	—	—
,, 104	—	—	—	—
,, 103 (*paracolon*)	—	—	—	—

TABLE XXVIII (*continued*).

Strains of	1 0·16 c.c.	2 0·22 c.c.	3 0·32 c.c.	4 0·42 c.c.
Type 106	—	—	—	—
„ 103. Developed lactose-fermenting mutant	++++	++++	++++	++++
„ 102. Developed lactose-fermenting mutant	++++	++++	++++	++++
„ 105. Lactose-fermenting mutant	++++	++++	++++	++++
„ 102. Lactose-fermenting mutant	++++	++++	++++	++++
„ 103. Lactose-fermenting mutant	++++	++++	++++	++++
Sub-group C.				
Type 1	++++	++++	++++	++++
„ 2	++++	++++	++++	++++
„ 5	++++	++++	++++	++++
„ 1	++++	++++	++++	++++
„ 2	++++	++++	++++	++++
„ 4	++++	++++	++++	++++
„ 3	++++	++++	++++	++++
„ 9	++++	++++	++++	++++
Sub-group D.				
Type 1	++++	++++	+++	+++
„ 3	—	—	—	—
„ 5	—	—	—	—
„ 7	++	—	—	—
„ 4	++++	+	—	—
„ 2	+	—	—	—

The strains of sub-group B showed some variation in their resistance to brilliant green, as might have been expected from the biological differences elicited by the serological tests.

Those belonging to the series 1–11 (fermenting lactose in primary culture) all showed a higher degree of resistance to the dye than the A types, and equal to or slightly greater than that of *B. typhosus*. Those types which developed lactose-fermenting mutants exhibited a high degree of resistance, *i. e.* much greater than that of series 1–11 or *B. typhosus*, while the paracolon types were apparently less resistant even than the A types.

In the B sub-group, therefore, the various types could be classified into three categories according to their resistance to brilliant-green correlated with certain cultural characters and reactions.

In the C sub-group all the strains tested exhibited a high resistance to brilliant green, equal to that shown by the mutating strains of sub-group B.

In the D sub-group there was some variation in the susceptibility of different types, but the number of strains available for testing was too limited to draw any inferences from the results.

These findings correlated with the serological observations are of considerable interest; from the serological study it was concluded that the gas +, indol +, inosite − types could be grouped together apart from the other organisms of the *B. coli* group. The tests carried out with these organisms growing on brilliant green media also show the striking distinction between organisms of the sub-group A on the one hand and the C types and also certain of the B types on the other.

While the A types (with few exceptions) are all more or less similar in their behaviour, and the same is also true for the C types, various B and D types behave differently.

In the B sub-group the lactose-fermenters were differentiated serologically from the paracolon varieties and those which only fermented lactose after mutation; in the brilliant green resistance tests a corresponding difference was established.

These experiments, therefore, apart from the practical bearing they had in connection with the brilliant green enrichment process for the isolation of *B. typhosus*, were of considerable interest in correlation with the previous work on the classification of the *B. coli* group.

VARIATION AMONG THE COLIFORM BACILLI.

Variation in Gas Production.

Among these organisms certain anomalies have been noted as regards this property (Mair, Wilson and others); thus strains when first isolated may show complete absence of gas production, but on repeated subculture develop this property. Reference has already been made to strains of paracolon bacilli (p. 329) which in primary culture simulated *B. typhosus* in their cultural reactions. The possibility of this variation must be considered, therefore, in the practical identification of intestinal bacilli. A *B. paratyphosus* A in the first cultures made after isolation may show complete absence of gas production, and if it only ferments dulcite slowly, as is often the case, it may thus simulate *B. typhosus* in cultural characters. *B. dysenteriae* Shiga may also be simulated by a non-motile organism which after repeated subculture ferments glucose with gas production, though in primary culture it produces no gas (of glucose, lactose, dulcite, saccharose, mannite, maltose, only glucose fermented).

My attention was first drawn to this variation by the occurrence of a strain of *B. coli* (B1), which, when tested shortly after isolation, produced gas from dulcite only, and when re-tested after a month's culture was found to have attained the power of producing gas from lactose and saccharose though still non-gas-producing in glucose. Later it also acquired the power of fermenting glucose with gas formation.

Though the absence of gas production in the case of *B. typhosus* and *B. dysenteriae* is known to be a stable character of these organisms, the question arose as to whether "*anaërogenes*" types of coliform bacilli simply represented variant strains of *aërogenes* types.

While certain strains have been noted which immediately after isolation produced no gas from *any* of the sugars fermented (*e. g.* B101), it was found that in many cases gas production was only absent in certain of the sugar tests (*e. g.* strain of B1 quoted above); also, as a general rule, such organisms after a few subcultures quickly developed the property of gas production, *i. e.* the character seemed to be only in abeyance. On the other hand, the various strains classified as "*anaërogenes*" (sub-group D), even after repeated subculture and after being kept in artificial growth for long periods, still remained non-gas-producing.

It has been shown by Penfold that by growing *B. coli* on monochloracetic acid agar a variant strain could be selected out which differed from the original in the absence of gas formation in certain sugars. With a view to determining the possibility of transmuting an *aërogenes* type into an *anaërogenes* variety, certain *B. coli* strains were submitted to Penfold's procedure.

Method.—The monochloracetic acid was made up in a 10 per cent. watery solution, and after having been made slightly alkaline to litmus by adding sodium carbonate, was sterilised by filtration through a Maassen filter. The solution was then incorporated in a 2 per cent. peptone-water-agar in measured proportions. The percentages indicated below are expressed in terms of the amount of the acid in the quantity of medium used for plating.

In the first experiment a series of plates each of 10 c.c. of monochloracetic-acid-agar, the acid being in the following proportions:

1	2	3	4
0·05 per cent.	0·1 per cent.	0·5 per cent.	1·0 per cent.

were inoculated with a typical *B. coli communis* strain. On plate 1 a normal amount of growth was obtained but the colonies varied considerably in size. On plate 2 the difference in the size of the colonies was more marked and many of the larger colonies showed papillae as described by Penfold. On plates 3 and 4 no growth appeared. A subcultivation on ordinary agar was made from a large colony on plate 2, and from this plates containing the following concentrations of monochloracetic acid were inoculated as before:

1	2	3	4	5
0·2 per cent.	0·3 per cent.	0·4 per cent.	0·5 per cent.	0·7 per cent.

On all these plates abundant growths were obtained and all the colonies were of the large type. A subcultivation was again made on ordinary agar from plate 5 and from this plates containing the acid in still higher proportions were inoculated.

1	2	3
0·5 per cent.	1 per cent.	2 per cent.

Growth was abundant on plates 1 and 2; in the case of plate 3 the growth was slower in appearing, but ultimately a few large colonies developed. Thus a monochloracetic acid resistant strain was selected out, and subcultures on ordinary medium when tested were found to produce acid only from glucose, acid and considerably reduced amount of gas from lactose and galactose, acid and gas (in a slightly reduced amount) from dulcite and mannite.

A similar test was carried out with a sub-group A type 1 strain; the selected strain capable of growing on 2·5 per cent. monochloracetic acid agar showed also absence of gas production in glucose, considerably reduced gas production in lactose and galactose, and slightly reduced gas production from dulcite and mannite. Other strains of typical *B. coli* and a *B. proteus* were tested with similar result as regards the particular sugars of the above series fermented.

The results differed from those of Penfold* in that the only "sugar" (of 2 monosaccharides, a disaccharide and 2 hexahydric alcohols) from which these variants completely failed to produce gas was glucose, though in the case of lactose and galactose there was some depression of the gas-producing property.

All these variants maintained their stability as regards the new character even after several months' subculture on ordinary agar, but by subculturing every day in glucose peptone water for a week, a reversion of the strain was noted and the power of producing gas was regained. Harden and Penfold found that, from the biochemical standpoint, the change was more a quantitative than a qualitative one.

Thus from my observations it was only possible to completely abolish the gas production of *B. coli* in the case of glucose, and it was also shown that the original character could be easily regained under certain conditions. It was in no way possible to select from an *aërogenes B. coli* type a corresponding *anaërogenes* variety.

The absence of gas production after several subcultures may, therefore, be regarded as a fundamental character of certain coliform types.

* Penfold's variant strains showed absence of gas production in lactose as well as glucose.

Such variations have been observed occurring spontaneously in culture medium, and afford some indication of the process of evolution going on among these organisms under natural conditions. The marked diversity of types in this group as regards cultural reactions has been well shown by the work of all those who have studied these organisms, and in the serological observations already recorded the highly specialised characters of individual strains have been alluded to; it may be assumed, therefore, that new types or species are constantly being developed from pre-existing varieties, and that these bacilli tend to acquire new characters which are not spontaneously lost, *i. e.* that the tendency is a progressive one and represents the origin of new species.

In 1907 Massini* described a non-lactose-fermenting Gram-negative bacillus (*B. coli mutabilis*) which on Endo-agar developed lactose-fermenting mutants represented by red papillae on the pale colonies of the original strain, and after further subculture as red colonies. This was corroborated by Burk and later by Müller in the case of the fermentation of other carbohydrates.

In studying the fermentative reactions of coliform bacilli it has been noted that certain strains may not show any obvious change in a particular sugar, *e. g.* lactose, until the lapse of several days; organisms of this type were investigated by Penfold, who showed that these slowly fermenting strains were primarily non-lactose-fermenters which in fluid media threw off lactose-fermenting variants, the variant producing rapid fermentation of the sugar. Thus the obvious difference between the variant and the original strain lay in the rate of fermentation of lactose. The strains studied were characterised by the formation on differential medium (such as MacConkey's neutral red lactose agar or Endo-agar) of pale colonies, which developed after a few days red papillae as in the case of Massini's strain.

A number of strains were met with in the course of my own study of the *B. coli* group which, though primarily non-lactose-fermenters, in fluid medium containing lactose apparently threw off lactose-fermenting variants. All these organisms formed pale colonies on MacConkey's agar; certain of them corresponded to the type described by Penfold in developing red papillae. By subculturing the papillae on another plate of MacConkey's medium, red colonies were grown representing a variant which differed from the original strain in producing rapid fermentation of lactose in fluid medium, whereas the sugar was only fermented after several days (and often without gas production) by the original strain.

Some of these organisms, on the other hand, showed no evidence of mutation on solid media containing lactose (*e. g.* MacConkey's agar), *i. e.* red papillae were not observed, but on subinoculating from the fluid lactose

* Reported also by Neisser (1906).

TABLE XXIX.—*In this table A = acid; G = gas, the amount of gas production being indicated by G+, G++, G+++; C = clot (from milk).*

Strain of type.	Days' incubation.	Lactose.	Dulcite.	Saccharose.	Adonite.	Milk.	
B102	1	—	—	—	—	—	Colonies on MacConkey's agar
	2	—	—	—	—	A	developed red papillae after
	5	A	—	—	—	AC	3 days.
Variant	1	AG+++	—	—	—	AC	
B104	1	—	—	—	—	A	Colonies on MacConkey's agar
	2	—	—	—	—	A	developed red papillae after
	5	AG+	—	—	—	AC	4 days.
Variant	1	AG+	—	—	—	AC	
D1	1	—	—	—	—	—	Absence of papilla formation.
	2	—	—	—	—	—	
	5	—	—	—	—	—	
	10	A	—	—	—	A	
Variant	1	A	—	—	—	A	
	2	A	—	—	—	AC	
B107	1	—	—	—	—	—	Absence of papilla formation.
	2	A	—	—	—	A	
	5	AG+	—	—	—	A	
Variant	1	AG+++	—	—	—	A	
	2	AG+++	—	—	—	AC	
A19	1	—	—	—	—	A	Absence of papilla formation.
	2	—	—	—	—	A	
	5	A	—	—	—	AC	
	10	AG+	—	—	—	AC	
Variant	1	AG++	—	—	—	AC	

Note.—In all 5 strains were noted corresponding in cultural reactions to B102 and also developing lactose-fermenting variants (represented by red papillæ on the colonies on MacConkey's agar).

TABLE XXIX (*continued*).

Strain of type.	Days' incubation.	Lactose.	Dulcite.	Saccharose.	Adonite.	Milk.	
A19	1	—	—	—	—	—	Absence of papilla formation.
	2	—	—	—	—	A	
	5	AG+	—	—	—	A	
Variant	1	AG++	—	—	—	AC	
B103	1	—	—	—	—	—	Absence of papilla formation.
	2	—	—	—	—	—	
	5	—	—	—	—	—	
	10	A	—	—	—	A	
Variant	1	AG++	—	—	—	AC	
B8	1	—	—	—	—	—	Absence of papillae from colonies on neutral red dulcite agar.
	2	—	—	—	—	—	
	5	—	—	—	—	—	
	10	—	A	—	—	—	
Variant	1	—	AG+	—	—	—	
B10 (after mutation)	1	—	—	—	—	—	Absence of papillae from colonies on neutral red adonite agar.
	2	—	—	—	—	—	
	5	—	—	—	—	—	
	10	—	—	—	AG+	—	
Variant	1	—	—	—	AG++	—	
B106	1	—	—	—	—	—	Absence of papilla formation on MacConkey's medium.
	2	—	—	—	—	—	
	5	—	—	—	—	A	
	10	A	—	—	—	A	
Variant	1	AG+	—	—	—	AC	
A28	1	—	—	—	—	—	Colonies on saccharose neutral red agar developed red papillae after 4 days.
	2	—	—	—	—	—	
	5	—	—	—	—	—	
	10	—	—	AG+	—	—	
Variant	1	—	—	AG++	—	—	

TABLE XXIX (*continued*).

Strain of type.	Days' incubation.	Lactose.	Dulcite.	Saccharose.	Adonite.	Milk.	
B8	1	—	—	—	—	—	Absence of papillae from colonies on saccharose neutral red agar.
	2	—	—	—	—	—	
	5	—	—	A	—	—	
Variant	1	—	—	AG+	—	—	
B105	1	—	—	—	—	—	Colonies on MacConkey's agar developed red papillae after 3 days.
	2	—	—	—	—	A	
	5	A	—	—	—	A	
	10	A	—	—	—	AC	
Variant	1	AG+	—	—	—	AC	
B103	1	—	—	—	—	—	Colonies on MacConkey's agar developed red papillae after 3 days.
	2	—	—	—	—	—	
	5	A	—	—	—	A	
	10	A	—	—	—	AC	
Variant	1	AG+	—	—	—	AC	
A34	1	—	—	—	—	—	Absence of papillae from colonies on MacConkey's agar.
	2	—	—	—	—	—	
	5	—	—	—	—	A	
	10	A	—	—	—	A	
Variant	1	AG+	—	—	—	A	
	2	AG++	—	—	—	AC	
A35	1	—	—	—	—	—	Absence of papillae from colonies on MacConkey's agar.
	2	—	—	—	—	—	
	5	A	—	—	—	A	
	10	A	—	—	—	A	
Variant	1	AG++	—	—	—	AC	
B101	1	—	—	—	—	—	Absence of papillae from colonies on saccharose agar.
	2	—	—	—	—	—	
	5	—	—	—	—	—	
	10	—	—	A	—	—	
Variant	1	—	—	AG	—	—	

medium in MacConkey's agar a mixture of pale and red colonies was obtained; the pale colonies corresponded to the primary strain, the red colonies to the variant.

Similarly, strains were noted which mutated as regards the fermentation of other sugars, *e. g.* dulcite, saccharose, adonite.

The variations exhibited by these strains and the differences between the original and the variant strain are shown in Table XXIX.

It is noteworthy that the majority of these mutating strains were of the B sub-group. While most of these variations were in the fermentation of lactose, it has been shown how similar mutations may occur in the fermentation of dulcite, saccharose and adonite.

It may also be noted here that no variations have been met with as regards the fermentation of inosite. Similarly the presence or absence of indol formation has been found to represent stable characters. Of course quantitative variations in indol production have been observed, and it has been shown how the amount of indol produced may be increased or diminished under certain conditions (Peckham, Horrocks). A considerable proportion of all the coliform strains examined were re-tested after two to three months from the time of their original classification, and in all cases indol formation or the absence of this property proved stable.

Variation in Cultural Characters Associated with Variation in Agglutinability.

It has been shown that variation in biochemical reactions is not associated with any change in the serological characters of the strain (Penfold) and this was confirmed in the case of one of the mutating strains described above (p. 339).

Variations in colony characters of certain *B. coli* strains produced by growth on an inhibitory medium (*i.e.* containing brilliant green) have, however, been found to be associated with considerable quantitative variation in agglutinability of the variant strains as compared with the original (Mackie).

It can be understood how a group of bacteria which are constantly developing mutants with new biochemical characters has come to represent in the course of time a considerable number of different cultural types as seen in the *B. coli* group; in the same way constant variation in serological characters, without change in biochemical reactions, would explain the highly specialised serological characters of the individual strain and the absence of specificity of an antiserum for the cultural type.

The process of evolution and origin of new types going on among these Gram-negative bacilli is not only of the greatest biological interest, but the study of these variations elicits some explanation of the great diversity of types and also the highly specialised serological characters of coliform bacilli.

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